





National Institute
of
Allergy and Infectious
Diseases

***Annual Report
of
Intramural Activities***

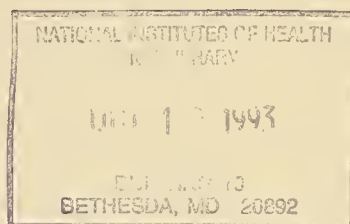
October 1, 1990-September 30, 1991

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

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of
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Annual Report of Intramural Activities

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U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

For Administrative Use

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1991 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI

00013-28 LIP
00020-16 ODIR
00027-24 LMM
00030-23 LI
00035-16 LI
00036-26 LI
00043-26 LCI
00045-23 LCI
00047-22 LHD
00048-21 LCI
00057-18 LCI
00058-17 LCI
00072-20 LPVD
00074-19 LPVD
00085-14 LPVD
00086-14 LPVD
00094-32 LPD
00097-33 LPD
00098-35 LPD
00099-21 LPD
00102-17 LPD
00108-18 LPD
00123-25 LVD
00126-18 LVD
00134-29 LI
00143-22 LIG
00144-27 LIG
00145-24 LIG
00154-16 LCI
00155-16 LHD
00161-14 LPD
00162-15 LPD
00166-14 LIG
00168-14 LIG
00169-14 BRB
00170-14 LIG
00171-14 LIG
00172-13 BRB
00180-13 LIG
00190-13 LMM
00193-12 LMSF
00197-12 LPD
00208-11 LPD
00210-11 LIR
00213-11 LIR
00216-11 LICP
00218-10 LMM

Z01-AI

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00241-10 LPD
00246-09 LPD
00248-10 LPD
00249-10 LCI
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00255-10 LPD
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00262-10 LPVD
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00343-10 LID
00345-10 LID

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1991 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-A1

00346-10 LID
00347-09 LPD
00349-09 LI
00350-09 LPD
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00353-09 LMM
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00356-09 LCI
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00370-09 LID
00372-09 LID
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00394-08 LI
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00415-07 LMM
00416-08 LVD
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Z01-A1

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00534-04 LID
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00538-04 LVD
00539-04 LVD
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00554-03 LVP
00557-03 LID
00558-03 LID
00562-03 LIR
00564-03 LVD

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1991 ANNUAL REPORT PROJECT NUMBER LISTING OF ACTIVE PROJECTS

Z01-AI

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00567-02 LICP
00568-02 LMSF
00569-02 LID
00570-02 LID
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00578-02 LIP
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00592-02 LCI
00593-02 LCI
00594-02 ODIR
00595-01 LCI
00596-01 LID
00597-01 LID
00598-01 LID
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00600-01 LID
00601-01 LID
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00608-01 LMSF
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00610-01 LPVD
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00614-01 LHD

Z01-AI

00615-01 LHD
00616-01 LID
00617-01 LCI
00618-01 LCI
00619-01 LVD
00620-01 LVD
00621-01 LCI

OFFICE OF THE DIRECTOR, DIVISION OF INTRAMURAL RESEARCH, NIAID
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SUMMARY OF PROGRAM
Laboratory and Clinical Research, NIAID
October 1, 1990 - September 30, 1991

The Division of Intramural Research (DIR), NIAID consists of 15 laboratories, a Biological Resources Branch, Administrative Management Branch and an Animal Care Branch. Four laboratories are located in Hamilton, Montana at the Rocky Mountain Laboratories (RML). The remaining facilities are concentrated at the NIH Bethesda Campus with auxiliary facilities at Frederick (Ft. Detrick) and Rockville, MD. The Office of the Director, DIR, (also called the Office of the Scientific Director, NIAID) is responsible for the management of the Division of Intramural Research as well as advising the Director, NIAID on scientific issues. In FY'91 the DIR, NIAID had an allocation of \$114,217. The allocation of the budget was as follows: 31.08% for salaries, 42.95% for laboratory support, 7.75% for the management fund and 18.01% for patient costs. In FY'91 there were over 865 persons working within DIR, NIAID with 93 tenured scientists, 604 nontenured scientists (including guest workers, special volunteers and summer students) and about 172 nonscientists.

Numerous outstanding accomplishments were made by DIR, NIAID scientists in FY'91. Some of the many highlights were as follows:

Vaccines to Prevent and Treat Infectious Diseases.

NIAID laboratories are actively engaged in developing new vaccines. Both classical and new methods are being employed. For example, in the classical approach microorganisms are being attenuated by applying molecular engineering techniques to modify protein determinants to render agents immunogenic but not pathogenic. This has been done for viruses such as dengue, influenza A, rotavirus, respiratory syncytial virus and hepatitis A and C. As a result promising candidate vaccines for these agents are, or soon will be, available for testing in animals and man. Studies of simian immunodeficiency virus (SIV) have resulted in a vaccine that protected monkeys from infection with free virus and the approach used for the SIV model will be evaluated for a HIV vaccine. In other studies a candidate HIV vaccine, based on purified gp160 envelope protein, is entering phase I clinical testing. A candidate vaccine for transmission-blocking immunity against *Falciparum* malaria has been developed based on a recombinant antigen (Pfs25) that was inserted into vaccinia. The vaccinia malarial antigen was capable of producing immunity in mice and monkeys and is now ready for phase 1 human trials. A vaccine for chlamydia, using the major outer membrane protein of chlamydia as the immunogen, has been developed and shown to protect monkeys from the ocular consequences of infection. This new chlamydia vaccine is ready to be assessed in animal models for protection against genital infection and hopefully will be available for phase 1 clinical studies in humans in the near future.

In other studies the vaccinia vector, developed in the NIAID intramural program, has been shown to be a particularly useful vector to package candidate vaccines. It is now possible to package multiple immunogens into the vaccinia vector and to thereby immunize against multiple infections with a single vaccine. A novel form of "intracellular" vaccination has been suggested by using parvovirus anti-sense vectors to induce intracellular resistance to HIV and herpes simplex virus. In other studies intracellular antibody mediated resistance to influenza virus was demonstrated.

Antiviral agents are being developed. NIAID scientists, in collaboration with NCI scientists, have developed a candidate human immunodeficiency virus therapeutic based on hybrid CD4-pseudomonas exotoxin that binds to virus as it buds from the cell surface and thereby kills virus infected cells. This CD4-immunotoxin is now entering phase 1 clinical testing. In other studies of antivirals, acyclovir was proven by NIAID investigators to suppress frequently recurring labial herpes. Studies in patients with HIV indicate that foscarnet blocks cytomegalovirus infection complicating AIDS.

A major problem in treatment of malaria is resistance to chloroquine. NIAID scientists have shown the resistance results from a gene product causing efflux of chloroquine from the parasite vacuole. The gene responsible for malaria resistance has been localized to chromosome 7 of the parasite and studies are now underway to isolate the gene. Characterization of the gene responsible for chloroquine resistance should enable design of new drugs to prevent resistance.

Enhancement of Host Defense and Control of Inflammation.

Cytokines are very important mediators of immunity and inflammation. Based largely on NIAID DIR studies, γ -interferon was licensed by the FDA for use in reducing infections in patients with chronic granulomatous diseases of childhood. Colony stimulating factors, which stimulate neutrophil production in bone marrow, were shown to be important therapy for management of the leukopenia of AIDS. In other studies, designed to delineate the mechanism by which important cytokines interact with cells, the chemoattractant interleukin-8 (Il-8) receptor was cloned and sequenced and candidate synthetic anti-inflammatory peptides that block the receptor are currently under study.

Gene therapy is an attractive approach for correcting congenital disorders and for modulating host defense. NIAID scientists have cloned and sequenced the genes for two forms of chronic granulomatous disease of childhood and successfully transfected one of the genes into a patient's B cells and achieved normal protein production. Numerous studies to develop strategies for gene therapy of CGD patients are underway.

Basic and Clinical Immunology.

On a very basic level of immunologic research, NIAID scientists have defined the conditions in which the T cell receptor and a co-stimulating signal interact to become inactivated (anergic); this has important implications for immune reactions, such as graft rejection. In studies of a mouse model of immunodeficiency NIAID scientists have discovered that retrovirus infection of B cells leads to B cell expression of a viral antigen known as a "superantigen" that stimulates a large family of T cells to produce mediators of immunodeficiency disease, such as Il-4 and Il-5. These studies are important because they suggest that retroviruses may be important causes of immune dysregulation in human diseases such as the autoimmunities.

Other basic immunology studies performed by NIAID scientists relate to allergic diseases. Recent studies have shown that Il-4 is absolutely essential to the regulation of B cell differentiation into IgE producing cells; thus Il-4 is the key lymphokine in allergic reactions. Il-4 and Il-5 were also shown to be necessary for the development of immune reactions that underlie the pathology of common parasite infections such as leishmanial and schistosomal infections. In this area of research it was shown that mast cells and basophils produce substantial amounts of Il-4 when they interact with IgE via IgE receptors. Thus there is a positive feedback loop in which Il-4 induces IgE and IgE in turn induces Il-4.

The effects of Il-4 on IgE may have direct implications for treating allergic diseases. Methods of directly blocking or counteracting the actions of Il-4 are being pursued as potential treatment of allergic reactions. In related investigations of immunologic mechanisms in allergy, it has been shown that mast cells produce inflammatory cytokines when mast cell IgE receptors interact with IgE. NIAID investigators have, in collaboration with NIAMS scientists, cloned and sequenced the IgE receptor on mast cells and methods to therapeutically regulate IgE receptor-IgE interactions are underway.

Extensive clinical immunology studies have been conducted recently at NIAID. For example, studies of the vasculitis syndromes have been extensive and NIAID is now a world center for the study of these diseases. Treatment strategies developed recently include the use of cytotoxic agents such as cytoxan and methotrexate, which have been shown to cure certain patients and produce prolonged remission in others. This past year NIAID scientists have discovered a new lymphoproliferative and autoimmune disease of a young woman that is strikingly similar to an autoimmune disease in a mouse animal model. In other studies NIAID clinical scientists have identified immunological abnormalities in patients with chronic E-B virus infection and in patients with the mysterious disease known as chronic fatigue syndrome. In relation to the latter it has been shown that patients frequently display an abnormal T cell phenotype characteristic of differentiated T cells; this strongly suggests that patients suffer from an occult infection which on the one hand causes T cell differentiation and on the other, chronic fatigue.

Studies of both the basic and clinical aspects of HIV infection receive extensive attention in the NIAID DIR. In addition to those studies mentioned above, studies range from molecular studies of the effects of cytokines on HIV expression in cells, to studies of the various cell surface molecules in cell-cell transmission of HIV, to studies of the mechanisms by which HIV kills CD4+ T cells and finally, to studies of the various aspects of the immune response to HIV and related retroviruses. These investigations interdigitate with studies in many NIH intramural programs and comprise an active program designed to find a better treatment of HIV disease, including studies of candidate AIDS vaccines, combination chemotherapy and exciting trials of new anti-retroviral drugs mentioned above.

Equal Employment Opportunity and Affirmative Action Programs have been given high level of attention this year. Our annual Introduction to Biomedical Research Program for Minority Students from across the U.S. and Puerto Rico was successful in providing students with a brief introduction to biomedical research. This year we initiated a close collaboration of our clinical program with Howard University Medical School and also started a new "Adopt a School"

program with Dunbar High School in Washington, D.C. The purpose of the latter was to reach out to young people and introduce them to the excitement and opportunities of biomedical science early in their education. This year in the annual minority summer program for high school, college and medical students, 81 students worked in NIAID laboratories; 43.2 percent of the students in the summer program were minorities.

In FY '91 several new appointments were made within the Division of Intramural Research, NIAID. Dr. Warren Strober was appointed Deputy Director, DIR; Dr. H. Clifford Lane was appointed Clinical Director and Dr. Stephen Straus was appointed Chief, Laboratory of Clinical Investigation. In addition, the Laboratory of Host Defenses (Dr. John I. Gallin, Chief) was established.

The NIAID Board of Scientific Counselors, under the leadership of Dr. Stanley Falkow, reviewed the Laboratory of Immunoregulation (Dr. Anthony S. Fauci, Chief) December 10-12, 1990, the Office of the Director, Division of Intramural Research (Dr. John I. Gallin, Director) February 11-12, 1991, the Laboratory of Immunology (Dr. William E. Paul, Chief) and the Biological Resources Branch (Dr. John E. Coligan, Chief) June 3-5, 1991. All of the reviews were excellent. This year Drs. Stanley Falkow, Rochelle Hirschhorn, and Dr. Zanvil Cohn completed their tenure on the Board and their devoted service was greatly appreciated. Dr. Dennis Kasper will be the new Chair of the Board.

In summary, in FY '91 the Division of Intramural Research of NIAID continues to be a vigorous scientific enterprise that leads the international scientific community in many areas of basic and clinical research in allergy and infectious diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00594-02 ODIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT

Structure and Function of Fcγ and Fcε receptors

PRINCIPAL INVESTIGATOR

Jean Pierre Kinet, Section Chief - MAIS, OSD, NIAID

Anna Teresa Brini, Visiting Assoc.

Martin Adamczewski, Visiting Fellow

Marie-Helene Jouvin, Visiting Assoc.

Kenichi Ochiai, Visiting Fellow

Helmut Kuster, Visiting Assoc.

Gai Mi Lee, Laboratory Worker

Li Zhang, Visiting Assoc.

Jami Willette-Brown, Microbiologist

Odile Mejan, Visiting Fellow

Fumiyoski Takizawa, Visiting Fellow

Rosella Paolini, Visiting Fellow

Bernie Effertz, IRTA

COOPERATING UNITS

-Laboratory of Clinical Investigation, NIAID (Rottem, Metcalfe)

-Laboratory of Experimental Immunology, NCI (O'Shea, Kennedy, Ortaldo)

-Cell Biology and Metabolism Branch, NICHD (Orloff, Klausner)

-John Hopkins University (McGlashan)

LAB/BRANCH

Division of Intramural Research

SECTION

Molecular Allergy and Immunology Section

INSTITUTE AND LOCATION

NIAID, Twinbrook II, NIH Rockville, MD 20852

TOTAL MAN-YEARS:

10.5

PROFESSIONAL:

10

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK

The attachment of the Fc region of immunoglobulins to receptors (FcRs) on many cells of the immune system triggers various functions such as phagocytosis, antibody-dependent cytotoxicity, and the secretion of potent mediators. One of these receptors (FcεRI), the high affinity receptor for immunoglobulin E (IgE) plays a central role in immediate allergic reaction. It is a complex structure of an IgE-binding α chain, a β chain and a homodimer of γ chains. Another of these receptors, the low affinity receptor for immunoglobulin G (FcγRIII) is also a multimeric complex which consists of an IgG-binding α chain and of γ chains identical to those found in FcεRI. During the past year, we have engaged in several types of studies involving the gene and subunit structure and the function of these two types of FcRs. These studies have yielded the following results: 1. We have cloned and expressed by transfection cDNAs encoding the human β chain of FcεRI. The complete genomic organization of the corresponding gene has also been characterized. 2. The promoter region of the human γ gene has been dissected into several regulatory elements, one of which is a positive element specific of hematopoietic cells. 3. By using the baculovirus system we can produce large amount of truncated α chain which contains the high affinity binding site for IgE. We also have expressed successfully the truncated α in glycosylation mutants of CHO cells. This material will be used to produce crystals. 4. We have found recently that the receptor subunits become phosphorylated on serine and tyrosine within 5 seconds of receptor engagement. This dual phosphorylation only affects the "activated receptors" and not the adjacent receptors which have not been engaged. Furthermore the receptors are immediately dephosphorylated upon receptor disengagement. 5. We have shown that FcγRIII in human NK cells associates with three different types of dimers, 'γ, γζ and ζζ. These dimers may be responsible for different signalling pathways. 6. We have discovered that mouse FcγRII and FcγRIII not only binds IgG with low affinity but also bind IgE with affinities comparable to IgG.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00020-16

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies with PolyICLC

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Hilton B. Levy, Ph.D. Special Assistant to the Scientific Director, NIAID

COOPERATING UNITS (if any)

Dr. Andre Salazar, Walter Reed; Col. Tom Monath, USAMRIID, Dr. J. Morales, Presbyterian Hospital, Puerto Rico; Dr. T. Balkan, WRAIR; Dr. Sam Baron, U. of Texas Med. School, Galveston, TX; Dr. M. Roy, USAMMDA; Dr. O. Morgan, U. of West Indies, Jamaica.

LAB/BRANCH

Office of the Scientific Director (OSD)

SECTION

INSTITUTE AND LOCATION

NIAID, NIH-Ft. Detrick, Frederick, Maryland 21702

TOTAL MAN-YEARS

1.7

PROFESSIONAL

1.0

OTHER

.7

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

With Dr. Andre Salazar, we are continuing our studies with PolyICLC in Multiple Sclerosis and Glioma. The M.S. patients appear to be stabilized in most cases, and in some are improved. Longer studies are needed. It is too soon to say anything about the Glioma patients. In the AIDS study with Drs. Salazar and Morales, it seems that the T4 counts become stabilized. Viral antigens decrease or disappear in a number of patients. We have started a cooperative study with Drs. Monath and Roy of USAMRIID and USAMMDA. As a first step in a trial of PolyICLC in prophylaxis in military personnel exposed to exotic viruses, a contract has been let to do a phase I dose escalation toxicity study in normal volunteers. A cooperative study is also underway with Dr. Tom Balkan of WRAIR. Studies have shown that drugs (or illnesses) that enhance immune system functions are frequently associated with changes in such neuro-behavioral functions as sleep, cognition, emotional state, and mental and physical performance levels.

Administrative duties with the Animal Care and Use Committee occupy a good portion of my time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00182-12 ODIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.C. Williams, Ph.D., Sr. Scientist, NIAID/ODIR, Chief, Intracellular Pathogens Branch, Bacteriology Division, USAMRIID

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director of Intramural Research Programs, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD and Bldg. 550, Ft. Detrick, Frederick, MD 21702-5011

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00183-12 ODIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Properties of Coxiella burnetii (Q fever) Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J.C. Williams, Ph.D., Sr. Scientist, NIAID, DIR, Chief, Intracellular Pathogens
Branch, Bacteriology Division, USAMRIID

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director of Intramural Research Programs, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda MD and Bldg. 550, Ft. Detrick, Frederick, MD 21702-5011

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated.

Animal Care Branch

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ANIMAL CARE BRANCH

1991 Annual Report

Summary

Much of the current research in the understanding and treatment of allergies and infectious diseases requires the use of laboratory animals. The care and treatment of these animals can have a profound affect on the results of experimental procedures. An animal's environmental conditions, its daily care, the presence or absence of disease-causing microorganisms, the amount of pain or distress that results from experimental manipulations all may affect the validity of research data. The NIAID intramural scientists today are very sensitive to these factors and their ethical responsibility for ensuring that animals used in research receive high quality animal care. All NIAID scientists conducting research that involves animals are required to attend the NIH course on "Using Animals in Intramural Research: Guidelines for Investigators." This course offers not only a broad perspective on current animal issues but also provides specific information on actual techniques and procedures.

It is the responsibility of the NIAID Animal Care Branch to provide daily care to all animals maintained within the NIAID animal facilities in Bethesda and Frederick, to oversee NIAID's intramural contracts involving animals, and to assist NIAID scientists with animal-related problems. The NIAID maintains five animal facilities at the NIH campus in Bethesda, Maryland; one animal facility in Rockville, Maryland; and two animal facilities at the Frederick Cancer Research Development Center in Frederick, Maryland. Approximately 36,000 square feet of space (26,000 sq. ft. at the NIH in Bethesda, MD; 3,500 sq. ft. at the FDRDC in Frederick, MD and 6,500 sq. ft. in Rockville, MD) are devoted to the NIAID Animal Care Program in Maryland.

The NIAID's Animal Care Branch provides guidance to the Institute's intramural scientists using animals in research projects. This guidance includes assistance in the purchase of animals, the selection and proper administration of anesthetics and analgesics, the diagnosis, treatment and control of infectious agents, and the performance of technical procedures in laboratory animals. The Branch maintains production colonies of over sixty different strains of mice, hamsters, cotton rats, and rabbits for NIAID investigators within DIR or contract animal facilities. Many of these animals are unavailable anywhere else in the world or are available only after long delays. The NIAID Division of Intramural Research is committed to the use of animals only when alternative methods are unavailable, and fully supports existing Federal rules and regulations pertaining to the care and use of animals in biomedical research. The Institute has three veterinarians to help manage its animal care and use program and has spent, in FY 91, over \$2,500,000 in upgrading its animal facilities and purchasing new animal equipment.

ADMINISTRATIVE MANAGEMENT BRANCH

ANNUAL REPORT FY 1991

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**ADMINISTRATIVE MANAGEMENT BRANCH, DIR
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1990 to September 30, 1991**

INTRODUCTION

The Division of Intramural Research (DIR) Administrative Management Branch (AMB) was established in June, 1986. The AMB provides the linkage between general administrative issues and scientific program management, development and priorities. The Branch is responsible for planning, directing and coordinating the administrative management functions for the DIR including personnel management, financial management, space management, procurement, and travel regulations. Each DIR laboratory and branch has its own group of administrative and procurement staff to provide day-to-day management and administrative assistance. The following is a brief exposition of accomplishments and activities during fiscal year 1991.

BUDGET AND FINANCIAL MANAGEMENT

In FY'91 the DIR budget allocation was increased by 6.2 percent over the FY'90 level, to a total budget of \$114,217,000. Forty percent was allocated for support of AIDS and AIDS related research. The AMB is responsible for the accounting and monitoring of these funds in each specific research category.

During FY'91, the new budget tracking system, Status of Funds (SOF), was further enhanced and developed for use within the program areas. This system immediately allows the AMB managers to access and analyze the financial data from the Division of Financial Management (DFM) directly on-line. The SOF has extensive flexibility and is able to produce several different types of financial reports to the laboratories to assist them in the management of their resources.

This system, in conjunction with the Administrative Management and Budget Information System (AMBIS) has allowed for better monitoring and understanding of in-house (S&SF) charges, which historically have been classified as "non-trackable." The on-line system is available to all NIAID Administrative Offices to support their respective laboratories and branches. In addition, AMBIS is available to all lab and branch staff in DIR, NIAID. The AMBIS has streamlined not only the Institute procurement process, but also the budget tracking and monitoring process. Both the AMBIS and SOF financial systems provide complete support for the DIR staff in the management of the budget.

The Twinbrook Facility gained control of the Facility Utilities and Lease tracking and obligations system from the NIH Space Management. This will provide a more accurate record of the actual expenses incurred and a more secure base for future projections of operating costs. With the relocation of the Laboratory of Immunogenetics from Building 4, the budget tracking and planning assistance process is now being administered by the Twinbrook Administrative staff.

RENOVATIONS AND SPACE RELOCATIONS

The AMB is responsible for studying space allocations in DIR, concentrating on flow of work as it relates to space assignments. Other responsibilities relative to space include insuring the adequacy of resources for renovations, coordinating laboratory renovations with the Division of Engineering Services (DES), budgeting of program and/or construction funds for such renovations, and meeting with the appropriate NIH officials to resolve problems. The Branch continued to play a major role in coordinating and monitoring the progress of many space renovations and relocations during FY'91.

Twinbrook II Facility

The DES phase 1 renovation at Twinbrook II has been completed. The renovation provides 15,000 sq feet of laboratory space for the Intramural research labs at Twinbrook. The renovation includes 1,640 sq. feet of BL-3 level space; 11,785 sq. feet of BL-2 laboratory space, and 7,500 sq. feet of BL-3 Animal Care Facility which will directly support the HIV and other studies for the laboratories.

The Phase 1A renovation to provide Library, Seminar, and office space in a 5,600 square foot area began in July. It is anticipated the Phase 1A design will be final in November 1991, with construction anticipated in March 1992.

The Phase 2 First Floor Laboratories renovation is proceeding with the final submission of the Program of Requirements (POR) anticipated in September. This renovation, 13,000 square feet, will provide four new laboratories with an average of 1,350 square feet per laboratory. The primary laboratory space will house a new X-Ray Crystallography Laboratory. In addition, an expansion of the animal facility will provide a walk-in-autoclave to meet the continuing needs of various species of "clean" rodents.

The Laboratory of Immunogenetics was relocated from Building 4 to 6,340 square feet of newly renovated laboratory space, consisting of three individual laboratory suites, in December 1990.

The Transmembrane Signalling Section, LI, was relocated from temporary laboratory space to permanent Twinbrook space in July.

The final move and permanent space for the Molecular Allergy and Immunology Section, DIR, was completed in July. This provides approximately 2,600 square feet of laboratory space.

Control Data Building

In July 1991, the Administrative Management Branch, DIR, relocated and centralized its entire procurement function at the Control Data Building (CDB).

Building 7

Building 7 has completely renovated its Animal Holding Facility in order to meet AAALAC accreditation. The Animal Care Branch successfully integrated its animal research into the Building 6B multi-user animal facility in March 1991.

Building 4

Space previously used for the on-site typing contract, Social and Scientific Systems (SSS), has been reassigned to the Laboratory of Viral Diseases. SSS now resides in the Building 4 library.

Members of the Viral Immunology Section and the Molecular Genetics Section, LVD, moved from the Twinbrook II facility to Building 4.

PROCUREMENT/CONTRACTS

Budget tracking and status reports with information for contract renewals, Board Reviews, and the Director, DIR Annual Contract Review was reassigned to the Financial and Management Services Section. This includes option year elections and modifications for all DIR Procurement contract support. This office also prepares all Statements of Work (SOW) and supporting materials, schedules Technical and Business evaluations for the renewal process for the Procurement Contracts, and all other support documentation required by the Division of Procurement Contracting Officer.

This year there were three renewal contract requests forwarded to the Division of Procurement. Of these, one has been advertised and an award is anticipated in early FY'92 with the two remaining to be awarded either mid or late FY'92.

In FY'91, AMB monitored and managed a contract portfolio totalling \$20.4 million consisting of 23 research and development contracts including PRI, 11 procurement contracts as well as several interagency agreements. Contract and budget tracking has been upgraded and enhanced to be able to provide more detailed information specifically for renewals and Board Reviews. These accounting reports work in concert with the SOF and AMBIS reports to provide monitoring of all aspects of the DIR contracting process.

A collaborative research project with the Malaria Section, Laboratory of Parasitic Diseases in Mali, Africa was initiated and is being administered by the AMB.

AUTOMATION

DIR automation support continues to grow and is provided through the contract mechanism and from the NIAID Information Technology and Evaluation Branch (ITEB). The DIR currently has nearly 700 LAN users. The Administrative Management and Budget Information System (AMBIS), was fully implemented throughout the Division in 1991. This system streamlines the

procurement process for scientists by providing guidelines for placing orders according to appropriate regulations, status reports on each request, automated budget tracking and access to NIH BPA listings. This system has provided procurement agents with automated order processing and financial monitoring. AMBIS has the ability to reconcile NIH's Division of Financial Management reports with AMBIS and DELPRO fiscal data. In 1991, AMBIS was installed at the Rocky Mountain Laboratories.

A clinical program instituted in 1990 to provide better tracking of clinical trials and protocols for AIDS patients continues to be improved and enhanced to serve multiple clinical uses. This program is a necessary tool for determining the cost of each active clinical protocol from inception to completion. This program gives the Institute a clearer picture of its clinical expenditures.

PERSONNEL

Staff of the Financial and Management Services Section are responsible for contract administration, financial management and budget execution, clinical research management, management analysis, automated systems and information planning. Members of the Operations Support Services Section are responsible for personnel, budget administration, procurement, program analysis, and day-to-day administrative support to the DIR. The Chief, AMB, continues to function in a dual capacity as Chief, AMB, DIR, and Chief of the Technology Transfer Branch, OAM, OD, NIAID.

All DIR Purchasing Agents were relocated to the Control Data Building in July 1991, and three new Lead Procurement Agent positions were created.



Biological Resources Branch
1991 Annual Report
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Annual Report
Biological Resources Branch
National Institute of Allergy and Infectious Diseases
October 1, 1990 to September 30, 1991

RESEARCH PROGRESS

The Biological Resources Branch (BRB) investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and hemopoietic differentiation. Special emphasis is placed on membrane receptors and differentiation antigens present on T and B lymphocytes, on the structures of major histocompatibility genes and proteins, on the molecular basis by which antigens are processed and presented to T cells, and on the *cis* elements and *trans* factors that regulate T cell receptor and class I gene expression. In addition to this fundamental immunological research, the BRB provides protein sequencing, peptide synthesis, and flow cytometry expertise and services for the DIR.

STUDIES ON THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules, as well as factors controlling the expression of class I and class II molecules are emphasized in this research.

Peptide Binding Specificity of Class I Molecules. To better understand the specificity of peptide binding by MHC class I molecules, the capacity of a panel of unrelated peptides to compete for the presentation of viral peptides presented by HLA-A3 and HLA-B27 was evaluated. The HIV-Nef7F peptide (74-82) was presented by HLA-A3 to Nef-specific HLA-A3-restricted CTL lines and the influenza nucleoprotein peptide NP (380-393) was presented by HLA-B27 NP(380-393)-specific HLA-B27-restricted CTL lines. These results were compared to studies from our group that have evaluated the capacity of this panel of peptides to inhibit presentation of an influenza nucleoprotein peptide (NP 335-349) by HLA-B37 and a matrix peptide (M1 57-68) by HLA-A2 to the appropriate peptide-specific CTL lines. Out of 41 peptides tested, only 5 bound to more than one of the MHC molecules analyzed. Pairwise comparisons of the peptide binding specificities among these four different class I molecules revealed no common competitor peptides in four of the six possible comparisons. Thus, each class I molecule appears to have a functionally distinct peptide binding site as reflected by the ability to bind largely non-overlapping sets of peptides.

Assembly of Class I Molecules Requires Peptide. The crystal structures of HLA-A2 and HLA-Aw68 reveal that the peptide-antigen forms an integral part of the HLA structure, being retained in a prominent groove even after purification and crystallization. It was shown that the heavy chain and β -2-microglobulin of HLA-A2, after separation and fractionation in denaturants, reassemble efficiently under renaturing conditions only in the presence of MHC-restricted peptides. A complex of heavy chain, β -2 microglobulin, and viral peptide in the ratio 1:1:1 is formed in up to 46% yield. Reconstitution is not stimulated by either of two peptides not restricted to HLA-A2. The reconstituted complex of HLA-A2 and the influenza virus (B/Lee/40) nucleoprotein peptide, NP (85-94), crystallizes under conditions previously used to crystallize HLA-A2. Peptide-linked folding and assembly suggests mechanisms for the unusual capacity of HLA to bind many peptides of diverse sequence.

Reconstitution of Class I Molecules from *E. coli* Expressed Proteins. The human Class I histocompatibility antigen HLA-A2 has also been assembled from subunits expressed separately in *E. coli*. A peptide that is known to be recognized by human cytotoxic T lymphocytes (CTLs) in association with HLA-A2 is a necessary component of the reconstitution mixture. The N-terminal extracellular fragment (about 275 amino acid residues) of the HLA-A2 heavy chain is initially synthesized as an insoluble aggregate. The aggregate is solubilized in denaturant, mixed with the influenza nucleoprotein 85-94 decapeptide (NP peptide), and diluted into a solution containing human β -2-microglobulin (β_2m), isolated from the *E. coli* periplasm. The HLA-A2 heavy chain becomes soluble in physiological solutions if both β_2m and the NP peptide are present. The reconstituted HLA-A2 complex is recognized by a monoclonal antibody that is specific for the native HLA-A2/ β_2m heterodimer, and is also recognized by a monoclonal antibody that recognizes β_2m .

Expression of the Liver-Specific Class I Gene, Q10. Transgenic mouse strains with either the wild type Q10 promoter or site-directed mutants of this promoter directing H-2D^d expression have been created. Using these mouse strains, we have begun to map genetic regions and identify factors important for the regulation of Class I gene expression.

MOLECULAR STUDIES ON T CELL RECEPTORS AND ACCESSORY MOLECULES

Diversity of γ/δ T Cell Receptors. Thirteen T cell hybridomas and two cell lines expressing $\gamma\delta$ TcR were generated from splenic T cells. At least three different types of γ -chains (V γ 2-C γ 1, C γ 2, and C γ 4) were shown to be expressed by these cells. Analysis of V δ gene expression revealed that V δ 5 gene segments are used by 53% of the cells in our panel. Other V δ segments expressed by our panel of cells were V δ 2, V δ 4, V δ 6, and V α 10, indicating that the V δ repertoire expressed in the spleen is similar, but possibly not identical to the adult thymus repertoire. Sequence analysis of the V-D-J joinings of the δ -chain messages revealed substantial diversity, indicating that the δ -chain repertoire expressed in peripheral lymphoid organs uses a significant portion of the potential diversity predicted for these chains.

Assembly of T Cell Receptors. The subunit interactions within the α/β and γ/δ T cell receptor-CD3 (TcR-CD3) complexes were compared. Both complexes contain at least seven chains $\alpha\beta$ and $\gamma\delta$ T cell receptor chains plus five CD3 chains ($\gamma\delta\epsilon\zeta$). It was found that in both TcR-CD3 complexes analogous subunit interactions could be defined: CD3 γ and CD3 δ each form a stable complex with separate CD3 ϵ chains. Both the TcR γ/δ and TcR α/β form stable receptor complexes with these CD3 γ/ϵ and CD3 δ/ϵ subunits. A ζ_2 homodimer is present in these TcR-CD3 complexes. Cross-linking results indicated that the TcR β and δ chains are each others structural homologs or that in TcR-CD3 complexes both TcR chains (α and β , γ and δ) are spatially closely associated with the CD3 γ chain. The identical subunit interactions in the α/β and γ/δ TcR-CD3 complexes indicate no large structural differences between these receptor complexes, but rather suggest a striking conservation of crucial interactions between the subunits in the TcR-CD3 complexes.

Vitronectin Receptor is a T Cell Activation Molecule. It was demonstrated that the T cell activation antigen, recognized by monoclonal antibody H9.2B8, is the murine homologue of the vitronectin receptor (VNR) and, thereby, provided initial evidence that VNR is expressed on lymphoid cells. VNR is expressed on a variety of cell lines, tumors, and Con A-activated splenocytes, but not resting T cells, and is capable of binding to the extracellular matrix proteins fibronectin, fibrinogen, and vitronectin, via the tripeptide sequence RGD. There is no evidence of novel β chains pairing with the VNR α chain, as has been demonstrated in some human cells. In view of recent studies demonstrating that this molecule functions as an accessory molecule in T cell activation, the VNR may play an important role in mouse T cell functions.

SYNTHESIS OF PEPTIDE ANTIGENS

During the past year, over 450 peptides have been produced by a PRI contract facility and within the Branch itself. These peptides have supported research projects in nearly all Laboratories of the NIAID. Synthetic peptides have been used to study the interaction of peptides with MHC class I and class II molecules. Such peptides have been used to define cytotoxic T lymphocyte or helper T cell epitopes of proteins from chlamydia, plasmodia, HIV, and influenza A and B as well as from heat shock proteins and myelin basic protein. Synthetic peptides have been used to prepare antisera against proteins originally described by gene cloning or protein sequencing methodologies. Such antisera have been prepared against proteins from the following sources: *Giardia lamblia*, respiratory syncytial virus, DR α and β chains, vaccinia, Friend virus, HIV, respiratory acquired infectious anemia virus, Leishmania proteins, IgE receptor, and Dengue virus. Peptides have also been used to define the functional regions of cytochrome b558, Leishmania proteins and a Herpes simplex virus leucine zipper containing protein.

Within the Branch, synthetic peptides were used to define peptidic epitopes from HTLV-I and HTLV-II proteins that were reactive with patient's sera. Multiple epitopes were defined from *env*, *gag* and *pol* encoded proteins, and one gp46 derived peptide allowed categorical distinction between HTLV-I and -II infections by ELISA. The SCL gene product, which is thought to be fundamentally important for hemapoietic differentiation, was described using an anti-peptide serum.

PROTEIN ANALYSES

Proteins from a large variety of sources have been sequenced in the facility. These include Herpes simplex virus I DNA replication protein (Fierer and Challberg, LVD), human

monocyte proteins (Kan, LCI), neutrophil NCF-3 cytosolic factor (Kwong and Leto, LCI), Plasmodium sexual stage proteins (Williamson and Kaslow, LPD), α glucosidase from Candida (Williamson, LCI), Vaccinia ECO 105 (Ahn, LVD), TGF β_3 (A. Roberts, NCI), and Duffy blood group antigen (Miller, LPD).

High performance liquid chromatography (HPLC) was utilized to demonstrate the production of endothelins by novel cell types. Primary rat astrocytes were shown to produce and release endothelin-3 as well as to possess receptors for endothelin -1, -2, and -3. These results indicated that astrocytes serve as a potential extravascular source of intracerebral regulation. The secretion of endothelin -1 and -3 by astrocytes was shown to be independently regulated. Further studies demonstrated that human macrophages synthesize and secrete endothelins -1 and -3 indicating that macrophage-derived endothelins may have an essential function in blood vessel physiology, and that aberrant production may contribute to vessel pathology.

FLOW CYTOMETRY ANALYSES

The Flow Cytometry Section has just acquired another multiparameter flow cytometer, a BD FACS 440, which will be incorporated into the facility as soon as time and finances allow. This instrument, which is capable of 5 parameter sorting and analysis, should ease the limitations on sorting time, which was a point of discussion at the Cell Sorter User's committee meeting held in June, 1991. Users in buildings 4, 7 and 10 are utilizing the networking services to analyze data acquired on all three multiparameter flow cytometers. A PC-base list mode analysis program has been installed on our network server and is now available to any of our users linked to the FCS network. The overall usage of FCS instrumentation has increased from FY '90, with a total of 3486 hrs. or approximately 3.5 hrs/day/instrument. This increase was due to an increase in sorting time of about 400 hours over the previous year.

Biological Resources Branch
Annual Report
October 1, 1990 to September 30, 1991

ADMINISTRATIVE REPORT

This period marks the fourth year of the existence of the Biological Resources Branch. During this period, a major reorganization of the Sections within the Branch was instituted. The Protein Analysis and Synthesis Section was created to incorporate the entire Molecular Structure Section and components of the former Synthetic Peptide Antigen Section. This newly created Section contains all equipment and personnel involved with providing research support for protein chemistry and synthetic peptides. Dr. Carl Hammer joined this Section as a transfer from the Laboratory of Clinical Investigation and Michael Knierman, a former employee of Program Resources Inc., was hired as Dr. Hammer's technician. Mr. Knierman has extensive experience in peptide synthesis and computer technologies. Mark Garfield continues to be responsible for protein sequence analyses and for making 25% of the synthetic peptides produced by the Branch. The Flow Cytometry Section continues to flourish under the able direction of Dr. Kevin Holmes and David Stephany. A fourth cell sorter/analyzer was recently acquired from NIH surplus by the Section and hopes are that it will be on line by the beginning of FY92.

On the research side, a new Molecular Immunology Section was created to include the entire basic research program of the Branch. It contains what was formerly contained within the Office of the Chief and the research component of the old Peptide Synthesis Section. The Office of the Chief now contains only functions pertaining to secretarial and administrative support for the Branch. In regard to research personnel, Dr. Angel Ezquerro departed to assume a research position in the Department of Immunology, Foundation of Jimenez Diaz, Madrid, Spain. Recent additions to the laboratory include Dr. Andrew Chang from the Department of Biochemistry, SUNY Health Science Center at Brooklyn and Dr. Marianne DiBrino from the Department of Immunology, University of Connecticut. Lisa Sestak was hired as a technician to replace Doug Markert. Dr. Yumiko Shirakata joined the Laboratory as a Special Volunteer from the Department of Molecular and Cell Biology, University of California at Berkeley.

**Biological Resources Branch
Annual Report
October 1, 1990 to September 30, 1991**

HONORS AND AWARDS

Dr. Coligan was invited to present research seminars at the Center for Disease Control in Atlanta and Howard University. He was also invited with expenses paid to speak at the Workshop on the Molecular Biology of Major Histocompatibility Complex Genes in Capri, Italy and at the FASEB meeting in Atlanta. Dr. Parker was a invited speaker at the FASEB summer conference on immunology. Dr. Coligan continues to serve on the editorial boards of the Current Protocols in Immunology, Molecular Immunology, and Immunologic Research. Dr. Coligan serves as Co-Chairperson on the Program Committee for the Major Histocompatibility Complex block for the AAI annual meeting. Dr. Coligan received the PHS Superior Service Award and Dr. Holmes has been nominated to receive the NIH Merit Award.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00169-14 BRB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan Branch Chief BRB/NIAID

Other: Yumiko Shirakata Special Volunteer BRB/NIAID

COOPERATING UNITS (If any)

LAB/BRANCH

Biological Resources Branch

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.9

PROFESSIONAL:

0.9

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

SUMMARY

We have created three transgenic constructs containing the gene encoding the MHC classical class I antigen, H-2D^d, linked to the MHC Q10 gene promoter. The first construct has the wild-type Q10 promoter. The other two constructs contain site-specific mutants restoring either the inverted repeat in CRE region 1 (single mutation) or the entire region 1 present in classical class I CRE gene promoters (two mutations).

RNase protection analysis has shown the presence of the transgene in the high levels in the liver of all three transgenic mouse strains. We have also observed low amounts of H-2D^d transcripts in the brain from all three lines, while transcription is absent from all splenic RNA. The wild-type promoter drives expression in the kidney while the mutant promoters fail to do so. FACS analysis has revealed expression of the transgene on CD4⁺CD8⁺ thymocytes in the mutant line that has the entire classical CRE region 1 binding site restored. This expression appears to be regulated in an age-related manner, as expression is virtually absent in three week old mice, but increases to a maximum level by approximately six weeks of age. In addition to the spleen, lymph nodes and bone marrow have been shown to lack H-2D^d, indicating that peripheral lymphocytes also lack expression of the transgenes. This observed regulation of transgenic expression in all three constructs is dissimilar from that of classical class I MHC gene expression.

We have identified liver-specific binding factors, including one that reacts with the TATA region of both Q10 and classical class I genes. Gel mobility shift assays have also demonstrated a specific factor from thymus extracts that binds to the classical class I CRE region 1 and one that binds to the enhancer B region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00172-13 BRB
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synthesis of Peptide Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) PI: John E. Coligan, Branch Chief BRB/NIAID Others: Carl Hammer, Senior Investigator BRB/NIAID		
COOPERATING UNITS (if any) CDC (Thomas Folks), NCI (Ilan Kirsch), NICHD (Keiko Ozato), Johns Hopkins (Jonathan Scheck).		
LAB/BRANCH Biological Resources Branch		
SECTION Protein Analysis and Synthesis		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: .3	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <div style="margin-top: 20px;"> <p>SUMMARY</p> <p>During the past year, over 450 peptides have been produced by a PRI contract facility and within the Branch itself. These peptides have supported research projects in nearly all Laboratories of the NIAID. Synthetic peptides have been used to study the interaction of peptides with MHC class I and class II molecules. Such peptides have been used to define cytotoxic T lymphocyte or helper T cell epitopes of proteins from chlamydia, plasmodia, HIV, and influenza A and B as well as from heat shock proteins and myelin basic protein. Synthetic peptides have been used to prepare antisera against proteins originally described by gene cloning or protein sequencing methodologies. Such antisera have been prepared against proteins from the following sources: <i>Giardia lamblia</i>, respiratory syncytial virus, DRα and β chains, vaccinia, Friend virus, HIV, respiratory acquired infectious anemia virus, Leishmania proteins, IgE receptor, and Dengue virus. Peptides have also been used to define the functional regions of cytochrome b558, Leishmania proteins and a Herpes simplex virus leucine zipper containing protein.</p> <p>Within the Branch, synthetic peptides were used to define peptidic epitopes from HTLV-I and -II proteins that were reactive with patient's sera. Multiple epitopes were defined from <i>env</i>, <i>gag</i> and <i>pol</i> encoded proteins, and one gp46 derived peptide allowed categorical distinction between HTLV-I and II infections by ELISA. The SCL gene product, which is thought to be fundamentally important for hemopoietic differentiation, was described using an anti-peptide serum.</p> </div>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00352-09 BRB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan Branch Chief BRB/NIAID

Others: Knut Sturmhoevel Visiting Fellow BRB/NIAID
 Gary Kikuchi Senior Staff Fellow BRB/NIAID
 Andrew Chang IRTA BRB/NIAID
 Scott Wadsworth IRTA BRB/NIAID
 Mark Halvorson IRTA BRB/NIAID
 Antonello Punturieri Visiting Assoc. BRB/NIAID

COOPERATING UNITS (If any)

University of Leiden, (Frits Koning)

LABORATORY

Biological Resources Branch

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

6.4

PROFESSIONAL:

5.7

OTHER:

.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Thirteen T cell hybridomas and two cell lines expressing $\gamma\delta$ TcR were generated from splenic T cells. At least three different types of γ -chains (V γ 2-C γ 1, C γ 2 and C γ 4) were shown to be expressed by these cells. Analysis of V δ gene expression revealed that V δ 5 gene segments are used by 53% of the cells in our panel. Other V δ segments expressed by our panel of cells were V δ 2, V δ 4, V δ 6, and V α 10, indicating that the V δ repertoire expressed in the spleen is similar, but possibly not identical to the adult thymus repertoire. Sequence analysis of the V-D-J joinings of the δ -chain messages revealed substantial diversity, indicating that the δ -chain a significant portion of the potential diversity predicted for these chains.

The subunit interactions within the α/β and γ/δ T cell receptor-CD3 (TcR-CD3) complexes were compared. Both complexes contain at least seven chains: $\alpha\beta$ and $\gamma\delta$ T cell receptor chains plus five CD3 chains ($\gamma\delta\epsilon\zeta_2$). It was found that in both TcR-CD3 complexes analogous subunit interactions could be defined: CD3 γ and CD3 δ each form a stable complex with separate CD3 ϵ chains. Both the TcR γ/δ and TcR α/β form stable receptor complexes with these CD3 γ/ϵ and CD3 δ/ϵ subunits. A ζ_2 homodimer is present in these TcR-CD3 complexes. Cross-linking results indicated that the TcR β and δ chains are each others structural homologs or that in TcR-CD3 complexes both TcR chains (α and β , γ and δ) are spatially closely associated with the CD3 γ chain. The identical subunit interactions in the α/β and γ/δ TcR-CD3 complexes indicate no large structural differences between these receptor complexes.

Evidence that the vitronectin receptor (VNR) is expressed on lymphoid cells was obtained. VNR was shown to be expressed on a variety of T cell lines, tumors, and Con A-activated splenocytes, but not resting T cells, and is capable of binding to the extracellular matrix proteins fibronectin, fibrinogen, and vitronectin, via the tripeptide sequence RGD. In view of recent studies demonstrating that this molecule functions as an accessory molecule in T cell activation, the VNR may play an important role in mouse T cell functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00522-04 BRB

PERIOD COVERED

October 1, 1990 - September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Sequence Analyses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: John E. Coligan Branch Chief BRB/NIAID

COOPERATING UNITS (if any)

LIR/NIAID (H. Ehrenreich), NCI (A. Roberts), London School of Hygiene and Tropical Medicine (J. Sachs), Roswell Park Memorial Institute (B. Seon)

LAB/BRANCH

Biological Resources Branch

SECTION

Protein Analysis and Synthesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

6

PROFESSIONAL:

.1

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

SUMMARY

Proteins from a large variety of sources have been sequenced in the facility. These include Herpes simplex virus I DNA replication protein (Fierer and Challberg,LVD), human monocyte proteins (Kan, LCI), neutrophil NCF-3 cytosolic factor (Kwong and Leto, LCI), Plasmodium sexual stage proteins (Williamson and Kaslow, LPD), α glucosidase from Candida (Williamson, LCI), Vaccinia ECO 105 (Ahn, LVD), TGF β_3 (A. Roberts, NCI), and Duffy blood group antigen (Miller, LPD).

High performance liquid chromatography (HPLC) was utilized to demonstrate the production of endothelins by novel cell types. Primary rat astrocytes were shown to produce and release endothelin-3 as well as to possess receptors for endothelin -1, -2, and -3. These results indicated that astrocytes serve as a potential extravascular source of intracerebral regulation. The secretion of endothelin -1 and -3 by astrocytes was shown to be independently regulated. Further studies demonstrated that human macrophages synthesize and secrete endothelins -1 and -3 indicating that macrophage-derived endothelins may have an essential function in blood vessel physiology, and that aberrant production may contribute to vessel pathology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00523-04 BRB

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Flow Cytometry Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kevin L. Holmes Head, Flow Cytometry Section BRB/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Resources Branch

SECTION

Flow Cytometry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.25

PROFESSIONAL:

.25

OTHER:

5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

The FCS has just acquired another multiparameter flow cytometer, a BD FACS 440, which will be incorporated into the facility as soon as time and finances allow. This instrument, which is capable of 5 parameter sorting and analysis, should ease the limitations on sorting time, which was a point of discussion at the Cell Sorter User's committee meeting held in June, 1991. Users in buildings 4, 7 and 10 are utilizing the networking services to analyze data acquired on all three multiparameter flow cytometers. A PC-base list mode analysis program has been installed on our network server and is now available to any of our users linked to the FCS network. The overall usage of FCS instrumentation has increased from FY '90, with a total of 3486 hrs. or approximately 3.5 hrs/day/instrument. This increase was due to an increase in sorting time of about 400 hours over the previous year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00543-04 BRB
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Recognition of Peptide Antigens by Virus-Specific Cytotoxic T Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: John E. Coligan Chief BRB/NIAID Others: Kenneth C. Parker Senior Staff Fellow BRB/NIAID Marianne DiBrino IRTA BRB/NIAID		
COOPERATING UNITS (if any) NINCDS (Bill Biddison); Harvard University (Don Wiley)		
LAB/BRANCH Biological Resources Branch		
SECTION Molecular Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <p>It was shown that the heavy chain and β-2-microglobulin of HLA-A2, after separation and fractionation in denaturants, reassemble efficiently under renaturing conditions only in the presence of MHC-restricted peptides. A complex of heavy chain, β-2 microglobulin, and viral peptide in the ratio 1:1:1 is formed in up to 46% yield. The reconstituted complex of HLA-A2 and the influenza virus (B/Lee/40) nucleoprotein peptide, NP (85-94), crystallizes under conditions previously used to crystallize HLA-A2. Peptide-linked folding and assembly suggests mechanisms for the unusual capacity of HLA to bind many peptides of diverse sequence. HLA-A2 has also been assembled from subunits expressed separately in <i>E. coli</i>. A peptide that is known to be recognized by human cytotoxic T lymphocytes (CTLs) in association with HLA-A2 is a necessary component of the reconstitution mixture. The HLA-A2 heavy chain only becomes soluble in physiological solutions if both β_2m and the NP peptide are present. The reconstituted HLA-A2 complex is recognized by monoclonal antibodies that are specific for the native HLA-A2/β_2m heterodimer. The isoelectric point of the reconstituted complex depends upon which peptide is used, confirming that the peptide is a component of the reconstituted complex.</p> <p>To better understand the specificity of peptide binding by MHC class I molecules, the capacity of a panel of unrelated peptides to compete for the presentation of viral peptides presented by HLA-A3 and HLA-B27 was evaluated. These results were compared to studies on the presentation of an influenza nucleoprotein peptide by HLA-B37 and a matrix peptide by HLA-A2 to the appropriate peptide-specific CTL lines. Out of 41 peptides tested, only 5 bound to more than one of the MHC molecules analyzed. Thus, each class I molecule appears to have a functionally distinct peptide binding site as reflected by their ability to bind largely non-overlapping sets of peptides.</p>		



Laboratory of Cellular and Molecular Immunology
1991 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Cellular and Molecular Immunology
October 1, 1990 through September 30, 1991

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. Its major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up *in vitro* analogs in tissue culture, and determine the molecular basis for the phenomena. The Laboratory employs techniques from the disciplines of cellular immunology, molecular biology, and protein biochemistry.

During its fifth year, the Laboratory has pursued four major areas of research: 1) T cell activation and inactivation, 2) the differentiation of T cell subsets, 3) positive and negative selection events occurring during T cell development, and 4) T cell tolerance and memory.

T Cell Activation and Inactivation

Studies of T cell activation of normal murine T cell clones of the T_H1 type have shown that two signals are required to stimulate the cells to make interleukin-2 (IL-2) and divide. One signal is given through the antigen-specific receptor that is uniquely expressed on each clone. The other signal is called costimulation and is delivered through a different receptor that has not yet been characterized. Signal 1 in the absence of signal 2 induces a state of hyporesponsiveness in the T cell clone known as clonal anergy. In this state, the cell fails to make significant amounts of IL-2 when restimulated with both signals 1 and 2. During the past year we have made progress in two areas. First, we have shown that the anergic state can be reversed in T cell clones by stimulating the cells with IL-2. This reversal occurs in all the cells and affects all impaired lymphokine production. Anergy was also found to dissipate spontaneously, although much more slowly, in the absence of IL-2 stimulation. Finally, we showed that a partial state of anergy could be induced by normal activation with antigen and APC provided that IL-2 and possibly other factors were removed from the culture medium by washing the cells. The results demonstrate that the anergic state is not a permanent change in the T_H1 cell. They also indicate that anergy induction might be a consequence of the inability of the cell to divide following stimulation. Thus, costimulation may only be required in normal activation to produce IL-2, which in turn prevents anergy by driving the cell through repeated rounds of division. In a separate set of experiments, we demonstrated that costimulation is required in order to make murine T_H1 clones competent to proliferate in response to interleukin-4. Induction of anergy in the cells blocked this ability to become competent in parallel with the impairment of the cells to make IL-2; however, IL-2 stimulation was not the only signal required to gain competence, because addition of IL-2 to a T cell-receptor occupancy signal, mediated by concanavalin A, only allowed the cells to become partially competent

to respond to IL-4. We concluded from these experiments that costimulatory signals contribute directly to the attainment of full competence to respond to IL-4.

(B. Beverly, S. Stringfellow, K. Brorson, K. Inamori, S. Umlauf, and R. H. Schwartz, LCMI, NIAID; S. Kang and M. Lenardo, LI, NIAID)

T Cell Subset Differentiation

Among the wide range of effector mechanisms that the immune system can use or recruit, it generally chooses only a restricted set of responses to ensure its defense against defined pathogens. Peripheral CD4⁺ and CD8⁺ T lymphocytes carry out these different functions during immune reactions, partly as a result of the distinct patterns of lymphokines that they secrete upon stimulation. By using thymic cells from adult and newborn mice, as well as from fetal thymic organ cultures, we showed during the past year that this functional differentiation occurs inside the thymus, and is completed during the single positive stage, by the time the T cell receptor becomes fully coupled to the intracellular activation pathways leading to lymphokine secretion. Surprisingly, CD4⁺8⁻ thymocytes differed from their immediate progeny, naive peripheral CD4⁺ cells, in that they secreted a broader spectrum of lymphokines, including IL-4, IL-5, IL-10, and γ -interferon, and in fact more closely resembled immunologically experienced (activated or memory) CD4⁺ lymphocytes. At least some of these cells represent newly developed thymocytes, and not recirculating cells, as they were generated in thymic organ cultures from 14 day fetuses. The frequency of IL-4 producing cells among CD4⁺8⁻, HSA low adult thymocytes was 1 in 20, and some of these cells were shown to emigrate from the thymus to the spleen over a 10 hr period. Our interpretation of these results is that the CD4⁺ thymocyte lineage goes through a previously undetected stage of development in which the cells can produce all the lymphokines characteristic of CD4⁺ memory T cells if they are stimulated through their T cell receptors. Subsequently the cells revert to a phenotype characteristic of virgin peripheral T cells which are only capable of making IL-2 on stimulation. Why CD4⁺ cells pass through this differentiation stage is not currently understood.

(A. Bendelac, D. Cassell, C. Prussin, H. Hashimoto, and R. H. Schwartz; LCMI, NIAID)

Positive and Negative Selection During T Cell Differentiation

These studies have attempted to deal with the paradox of how signaling through a given TCR (T cell antigen receptor) can mediate both positive and negative selection. Several of our investigations suggest that the affinity of the interaction determines the developmental outcome. In male mice which express both transgenic CD8 and anti-HY TCR, cells bearing the transgenic TCR but low levels of endogenous CD8 are clonally deleted, whereas the CD8 transgene does not lead to deletion of the CD4⁺8⁻ TCR⁺ cells. The results suggest that the mice possess both deletable and nondeletable lineages. The CD8 transgene also affects the ultimate fate of T cells developing in anti-L^d TCR transgenic mice. In H-2^b mice, where only positive selection is evident, introduction of the CD8 transgene results in negative selection. Since the CD8 coreceptor strengthens the TCR-MHC interaction, it would appear that a change in affinity controls the developmental outcome. Additional results suggest that positive and negative selection may not be ordered. If appropriate

chimeras are constructed, such that positive selection cannot occur, clonal deletion is still observed. This result rules out a maturational requirement (as a result of positive selection) in order for negative selection to occur. Using two transgenic mouse systems, one with the class I-restricted TCR, and one with a class II restricted TCR, antigen presentation was targeted to only the thymic epithelium by using radiation/bone marrow chimeras. In both of these systems, the thymic epithelium is able to mediate clonal deletion of the transgenic receptor-bearing T cells. Since epithelial cells can mediate both positive and negative selection events, these results also support a model of development that invokes differential signaling through different strengths of interaction. Finally, the thymus is also able to maintain tolerance to self antigens by a nondeletional mechanism which was observed when the antigen was expressed only on the epithelium. Adoptive transfers of the nonresponsive cells into hosts that lack the relevant self antigen results in a reversal of the nonresponsive state. Thus, persistence of antigen is required to maintain this nondeletional form of *in vivo* tolerance.

(F. Ramsdell, E. Robey, and B. J. Fowlkes, LCMI, NIAID)

T Cell Tolerance and Memory

The half life of virgin and memory T cells has been studied in the presence and absence of antigen. Female mice, thymectomized at 6 weeks of age, retain the ability to respond to the male antigen, H-Y, for about 20 weeks. Memory cells can also rest for some time in the absence of antigen, but, contrary to current dogma, we find that they do not last indefinitely. Primed T cells, transferred into adoptive hosts without a source of antigen, lose their ability to respond to KLH or H-Y in about 12 weeks, though they can respond for 8 months if transferred with antigen. We conclude from this that long term memory is not due to long lived cells but to the long term persistence of antigen. Thus the resting lifespan of virgin and memory T cells appears to be similar. We have also found that virgin and experienced T cells differ in their ability to respond to antigen presented by B cells. SCID mice which have been grafted with fetal thymuses are easily primed for proliferative responses to protein antigens. Thus, B cells are not necessary for priming. We have, in fact, found that antigen presentation by B cells is tolerogenic for virgin T cells. Unprimed female mice, injected with purified resting or activated male B cells become permanently tolerant of H-Y, while previously primed females are boosted by similar injections. Thus virgin T cells are turned off by recognition of antigen on a B cell whereas experienced T cells are turned on, suggesting that different co-stimulatory molecules are involved for the two T cell types. The tolerogenic effect of antigen presentation by B cells may explain high and low zone tolerance as well as the transplant enhancing effect of blood transfusions. Finally, the functional role of thymic epithelium is not totally clear. In addition to its role in positive selection of immature T cells, thymic epithelium is reported to induce a form of split tolerance. We have investigated this and found that thymic epithelium is absolutely tolerogenic for maturing T cells and that the tolerance induced is specific for the antigens expressed by thymic epithelium. The examples of split tolerance occur when tolerant T cell populations are tested for reactivity to other organs such as spleen or skin. Thus, the tolerance induced by thymic epithelium is not split tolerance, but simply a case of tissue specific tolerance.

(A. Bonomo, E. Fuchs, and P. C. E. Matzinger, LCMI, NIAID)

Administrative, Organizational, and Other Changes

During the past year Dr. Scott Umlauf joined the laboratory and Drs. Ephraim Fuchs, Hiro Hashimoto, Calman Prussin, and Steven Stringfellow left the laboratory.

Honors, Awards, and Scientific Recognition

Dr. Schwartz is a member of the editorial boards of *Science*, *International Immunology*, *Immunology Today*, *The Journal of Molecular and Cellular Immunology*, and *The International Journal of Cell Cloning*. This year he was awarded the Distinguished Service Medal by the U.S. Public Health Service.

During the past year he was a symposium speaker at the Chicago Association of Immunologists 25th anniversary meeting in Chicago, IL, a guest speaker at the 26th annual meeting of the Japanese Society for Transplantation in Okayama, Japan; a symposium speaker at the Nature meeting in Boston, MA; Co-chairperson of the Midwinter Conference of Immunologists in Asilomar, CA; a symposium speaker at the 1991 Keystone Symposium on Self Reactivity and its Regulation at Keystone, CO; a symposium speaker at the conference on the Role of T Cell Subsets and Cytokines in the Regulation of Infection held in Bethesda, MD; and a session chairman and speaker at the FASEB conference on Lymphocytes and Antibodies held in Saxton's River, VT. He has been invited to give research seminars at Stanford University, Stanford, CA; the University of California at Berkeley, Berkeley, CA; Thomas Jefferson University, Philadelphia, PA; the Barbara Davis Center for Childhood Diseases at the University of Colorado Health Sciences Center, Denver, CO, and the Fox Chase Cancer Center, Philadelphia, PA. Finally, he was again a lecturer in the American Association of Immunologists advanced course in Immunology in Colorado Spring, CO.

Dr. B. J. Fowlkes was appointed to the Committee on the Status of Women of the American Association of Immunologists. During the past year, she was an invited chairperson and speaker for a workshop on the Generation and Selection of the T cell Repertoire held at the NIH Research Festival '90; an invited symposium chairperson and speaker at the XVI New England Immunology Conference in Woods Hole, Massachusetts; an invited symposium speaker at the 11th Annual Meeting of the American Society for the Immunology of Reproduction in Charlottesville, Virginia; an invited symposium speaker at the Thirtieth Midwinter Conference of Immunologists on Tolerance and Immune Regulation held in Pacific Grove, California; a guest lecturer for the Genetics Course on T Cell Receptors at the University of Michigan in Ann Arbor, Michigan; and an invited chairperson for the T Cell Tolerance Workshop at the FASEB Society meeting in Atlanta, Georgia. Dr. Fowlkes has also been invited to give research seminars at George Washington University in Washington, DC; Cornell University Veterinary School in Ithaca, New York; New York University Medical Center in New York City; Memorial Sloan-Kettering Cancer Center in New York City; State University of New York Health Sciences Center in Syracuse, New York; Columbia University in New York City; and the University of Chicago in Chicago, Illinois.

Dr. P. C. E. Matzinger was an invited speaker at the Nature meeting in Boston, MA; CASW's 28th Annual New Horizons of Science Briefing in Philadelphia, SIPI's "TV News: The Cutting Edge", in Chicago, and the FASEB Conference on Lymphocytes and Antibodies, Saxton's River, VT. She has been invited to give research seminars at the University of Toronto, Ontario, Canada, Northwestern University Medical School, Chicago, IL, and the University of Maryland Immunology group, Baltimore, MD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT			PROJECT NUMBER Z01 AI 00485-05 LCMI
PERIOD COVERED October 1, 1990 to September 30, 1991			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) T Cell Activation			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	Ronald H. Schwartz	Chief	LCMI, NIAID
Others:	Bart Beverly	Staff Fellow	LCMI, NIAID
	Steven Stringfellow	ROG	LCMI, NIAID
	Kurt Brorson	IRTA	LCMI, NIAID
	Ken Inamori	VF	LCMI, NIAID
	Scott Umlauf	IRTA	LCMI, NIAID
COOPERATING UNITS (if any) Sang-Mo Kang and Michael J. Lenardo, LI, NIAID			
LAB/BRANCH Laboratory of Cellular and Molecular Immunology			
SECTION T Cell Activation Section			
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892			
TOTAL MAN-YEARS:	7.5	PROFESSIONAL:	5.5
		OTHER:	2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> Studies of T cell activation of normal murine T cell clones of the T_H1 type have shown that two signals are required to stimulate the cells to make interleukin-2 (IL-2) and divide. One signal is given through the antigen-specific receptor that is uniquely expressed on each clone. The other signal is called costimulation and is delivered through a different receptor that has not yet been characterized. Signal 1 in the absence of signal 2 induces a state of hyporesponsiveness in the T cell clone known as clonal anergy. In this state, the cell fails to make significant amounts of IL-2 when restimulated with both signals 1 and 2. During the past year we have made progress in two areas. First, we have shown that the anergic state can be reversed in T cell clones by stimulating the cells with IL-2. This reversal occurs in all the cells and affects all impaired lymphokine production. Anergy was also found to dissipate spontaneously, although much more slowly, in the absence of IL-2 stimulation. Finally, we showed that a partial state of anergy could be induced by normal activation with antigen and APC provided that IL-2 and possibly other factors were removed from the culture medium by washing the cells. The results demonstrate that the anergic state is not a permanent change in the T_H1 cell. They also indicate that anergy induction might be a consequence of the inability of the cell to divide following stimulation. Thus, costimulation may only be required in normal activation to produce IL-2, which in turn prevents anergy by driving the cell through repeated rounds of division. In a separate set of experiments, we demonstrated that costimulation is required in order to make murine T_H1 clones competent to proliferate in response to interleukin-4. Induction of anergy in the cells blocked this ability to become competent in parallel with the impairment of the cells to make IL-2; however, IL-2 stimulation was not the only signal required to gain competence, because addition of IL-2 to a T cell-receptor occupancy signal, mediated by concanavalin A, only allowed the cells to become partially competent to respond to IL-4. We concluded from these experiments that costimulatory signals contribute directly to the attainment of full competence to respond to IL-4. </p>			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00613-01 LCMI
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) T Cell Subsets		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)		
PI:	Ronald H. Schwartz	Chief LCMI, NIAID
Others:	Albert Bendelac	VF LCMI, NIAID
	Delanie Cassell	IRTA LCMI, NIAID
	Calman Prussin	ROG LCMI, NIAID
	Hirofumi Hashimoto	HHMS LCMI, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Cellular and Molecular Immunology		
SECTION T Cell Activation Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)		
<p>Among the wide range of effector mechanisms that the immune system can use or recruit, it generally chooses only a restricted set of responses to ensure its defense against defined pathogens. Peripheral CD4⁺ and CD8⁺ T lymphocytes carry out these different functions during immune reactions, partly as a result of the distinct patterns of lymphokines that they secrete upon stimulation. By using thymic cells from adult and newborn mice as well as from fetal thymic organ cultures, we showed during the past year that this functional differentiation occurs inside the thymus, and is completed during the single positive stage, by the time the T cell receptor becomes fully coupled to the intracellular activation pathways leading to lymphokine secretion. Surprisingly, CD4⁺8⁻ thymocytes differed from their immediate progeny, naive peripheral CD4⁺ cells, in that they secreted a broader spectrum of lymphokines, including IL-4, IL-5, IL-10, and γ-interferon, and in fact more closely resembled immunologically experienced (activated or memory) CD4⁺ lymphocytes. At least some of these cells represent newly developed thymocytes, and not recirculating cells, as they were generated in thymic organ cultures from 14 day fetuses. The frequency of IL-4 producing cells among CD4⁺8⁻, HSA low adult thymocytes was 1 in 20, and some of these cells were shown to emigrate from the thymus to the spleen over a 10 hr period. Our interpretation of these results is that the CD4⁺ thymocyte lineage goes through a previously undetected stage of development in which the cells can produce all the lymphokines characteristic of CD4⁺ memory T cells if they are stimulated through their T cell receptors. Subsequently the cells revert to a phenotype characteristic of virgin peripheral T cells which are only capable of making IL-2 on stimulation. Why CD4⁺ cells pass through this differentiation stage is not currently understood.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00486-05 LCMI
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) T Cell Differentiation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B.J. Fowlkes	Senior Investigator LCMI, NIAID
Others:	F. Ramsdell E. Robey	NIH Staff Fellow Guest Researcher LCMI, NIAID LCMI, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Cellular and Molecular Immunology		
SECTION Section on Thymocyte Differentiation		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>These studies have attempted to deal with the paradox of how signaling through a given TCR (T cell antigen receptor) can mediate both the positive and negative selection events that occur during T cell development. Several of our investigations suggest that the affinity of the interaction determines developmental fate. In male mice which express both transgenic CD8 and anti-HY TCR, cells bearing the transgenic TCR but low levels of endogenous CD8 are clonally deleted, whereas the CD8 transgene does not lead to deletion of the CD48⁺ TCR⁺ cells. The results suggest that the mice possess both deletable and nondeletable lineages. The CD8 transgene also affects the ultimate fate of T cells developing in anti-L^d TCR transgenic mice. In H-2^b mice, where only positive selection is evident, introduction of the CD8 transgene results in negative selection. Since the CD8 coreceptor strengthens the TCR-MHC interaction, it would appear that a change in affinity controls the developmental outcome.</p> <p>Additional results suggest that positive and negative selection may not be ordered. If appropriate chimeras are constructed, such that positive selection cannot occur, clonal deletion is still observed. This result rules out a maturational requirement (as a result of positive selection) in order for negative selection to occur.</p> <p>Using two transgenic mouse systems, one with a class I-restricted TCR, and one with a class II restricted TCR, antigen presentation was targeted to the thymic epithelium only by using radiation/bone marrow chimeras. In both of these systems, the thymic epithelium is able to mediate clonal deletion of the transgenic receptor-bearing T cells. Since epithelial cells can mediate both positive and negative selection events, these results also support a model of development that invokes differential signaling through different strengths of interaction.</p> <p>The thymus is able to induce tolerance to self antigens by a nondeletional mechanism which was observed when the relevant antigen is expressed only on the epithelium. Adoptive transfers of the nonresponsive cells into hosts that lack the self antigen results in a reversal of the nonresponsive state. Thus, persistence of antigen is required to maintain this nondeletional form of <i>in vivo</i> tolerance.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00581-02-LCMI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

T Cell Memory and T Cell Tolerance

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. Matzinger Expert LCMI, NIAID

Others: A. Bonomo VF LCMI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Tolerance Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

We have devised a new method to measure cytotoxic T cell activity *in vitro* and perfected a method to measure immune rejection *in vivo*. Using these techniques, in combination with our standard thymic organ culture, we are in the process of studying four questions.

1. Do newly born T cells leave the the thymus in a tolerizable or activateable state.

2. Does thymic epithelium present antigens in a tolerogenic or immunogenic fashion.

3. Does T cell priming and/or memory require the presence of B cells.

4. What is the frequency of alloreactive T cells.

Our preliminary experiments suggest that newly developed CD4⁺ and CD8⁺ T cells are fully capable of responding to allogeneic stimulators, thus refuting models that require the assumption that T cells leave the thymus in an anergizable state.

LABORATORY OF CLINICAL INVESTIGATION
1991 ANNUAL REPORT
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Summary of Report
LABORATORY OF CLINICAL INVESTIGATION
October 1, 1990 to September 31, 1991
Dr. Stephen E. Straus, M.D.
Chief, Laboratory of Clinical Investigation
NIAID

Introduction:

This has been an unusually eventful and promising year for the Laboratory of Clinical Investigation. Just prior to the start of the year, Dr. Michael Frank ended his long and productive tenure as Laboratory Chief. He left to become Chairman of the Department of Pediatrics at Duke University with all of the Laboratory's gratitude for a job well done and wishes. Dr. Frank left the Laboratory in a strong position, with eminent scientist remaining at the helm of all its Sections.

Dr. Stephen Straus, Head of the Medical Virology Section in the Laboratory, was appointed in March to assume the position of Laboratory Chief. Concurrently, and thereafter, additional actions pertinent to the structure and resources of the Laboratory were made.

First, the position of Clinical Director, that Dr. Frank and previous Laboratory Chiefs also occupied, was delineated as a separate administrative entity and assigned to Dr. H. Clifford Lane. This afforded the resources and emphasis that the NIAID clinical programs warrant. At the same time, the action withdrew from the Laboratory of Clinical Investigation personnel pertinent to the Clinical Director's charge.

Second, Dr. John Gallin created and assumed leadership of the new Laboratory of Host Defenses. He, Dr. Malech and all of their colleagues and resources, formerly of the Bacterial Diseases Section of the Laboratory, were assigned to this new Laboratory.

Third, the Clinical Immunology Section of the Laboratory was terminated. With the departure of Dr. Frank, its head, several other staff reassignments were made. Dr. Carl Hammer moved to occupy a senior position in the Biological Resources Branch. Ms. Thelma Gaither moved to assist in the Antiviral Research Branch. Dr. Straus established the Bacterial Pathogenesis Unit under the leadership of Dr. Thomas Russo.

There has been a substantial reallocation of laboratory space as well. Areas previously assigned to Dr. Frank were reassigned to Dr. Straus for the Office of the Chief and afforded expansion for the Medical Virology Section. The former space of the Medical Virology Section was assigned to the Laboratory of Host Defenses. The Gene Therapy Unit was absorbed into the Laboratory of Host Defenses and its space was reassigned to Dr. Russo.

Under Dr. Straus's leadership additional changes have begun to be made. The administrative association between the Clinical Parasitology Section and the Laboratory of Clinical Investigation was ended so as to incorporate the Section fully into the Laboratory of Parasitic Diseases. Dr. Michael Sneller, formerly a Medical Staff Fellow in the Mucosal

Immunity Section was converted to a Medical Officer, with the charge of establishing an aggressive clinical research program allied to the existing basic research endeavors of the Laboratory, particularly that of the Mucosal Immunity Section. Dr. Sneller will work on various aspects of immunomodulatory treatments for infectious, allergic, and immune diseases.

Dr. Stephen James, a Senior Staff Fellow in the Mucosal Immunity Section departed in the Spring of 1991 to become Professor and Head of the Division Gastroenterology at the University of Maryland. Dr. Robin McKenzie, a Medical Staff Fellow in Dr. Frank's Section, was converted to a part-time Medical Officer to assist Dr. Straus in his clinical research projects.

In all, the changes in the Laboratory of Clinical Investigation has reduced its size by several budgeted positions and a loss of about 500 square feet of laboratory space. The remaining and committed resources, however, are generous as to provide outstanding and stable support for the existing sections as well as expectations of growth and programmatic initiatives.

Research Progress

The **Medical Virology Section** has had an outstanding year despite the challenges of administrative and physical changes. Dr. Ostrove departed the NIH at the end of the last fiscal year. His efforts have been subsumed under the personal direction of Dr. Straus and by Dr. Jeffrey Cohen, a new Senior Staff Fellow recruited from Harvard University where he was a junior faculty member in Infectious Diseases.

The Section's emphasis continues to be on basic and clinical aspects of herpesvirus biology and pathogenesis. Important progress has been made in the treatment and prevention of herpes simplex virus infections. With collaborators in the Dental Institute, Dr. Straus has shown in controlled trials that acyclovir and sunblockers effectively prevent ultraviolet light-induced reactivation of herpes and that chronic oral acyclovir therapy effectively suppresses frequently recurring orolabial herpes. These are the first studies to clearly delineate effective suppressive treatment strategies for that disease.

In a related vein, the first trials of a recombinant glycoprotein vaccine for genital herpes have begun in the Section. The vaccine has proven to be extremely immunogenic and well tolerated. Controlled trials of its use for immunotherapy of recurrent genital herpes began this year.

The molecular biology of herpesvirus latency and gene expression is the major focus of the Section's basic research thrust. The Section continues to explore the structure and control of the herpes simplex latency genes. This year the corresponding gene of herpes simplex virus type 2 was identified, cloned, sequenced and analyzed. Efforts to discern its impact on the high rate of genital herpes recurrences are under way.

This Section leads in the delineation of the nature and mechanisms of latent varicella-zoster virus infection in humans. During the past year, a new in vitro latency model was

established and a refined hybridization system permitted the characterization of specific regulatory and early gene products that are expressed in latency.

Dr. Cohen has assumed the responsibility of furthering the Section's work on varicella-zoster virus regulatory genes and is doing so by creating families of insertionally-deleted viruses and corresponding cell lines stably transformed to express each one. Dr. Cohen has also brought to the laboratory his expertise in Epstein-Barr virus molecular biology and is pursuing analysis of the structure and function of the EBNA-2 gene. He has proven that a discrete domain of this gene's product is necessary for transformation of B cells.

The Section has identified a new mechanism whereby HIV transcription can be activated; this through studies of the effects of herpes simplex virus immediate early genes on HIV replication in lymphocytes. It appears that herpes genes markedly stimulate the activity of a novel cell protein that binds to the HIV transcription start site. The gene for this protein is being cloned from a Jurkat cell cDNA library.

The Section continued multidisciplinary collaborative studies of Chronic Fatigue Syndrome during the past year; several new findings were made. First, a novel neuroendocrine defect was identified through controlled studies of hypothalamic-pituitary-adrenal axis function. Second, in comparison with healthy controls, patients with the Syndrome were found to have shifts in lymphocyte subpopulations and in vitro responsiveness, in patterns indicating subtle but clear immune activation.

The **Clinical Immunology Section** continued to carry forward a number of projects over the past year despite the departure of its head Dr. Michael Frank. By spring 1991, all but two of the projects were terminated, one was consolidated under the aegis of a new project number and moved to the Biological Resources Branch under the direction of Dr. Carl Hammer. This project entails the characterization of an abundant 120 kd serum glycoprotein. During the past year sequencing of clones selected from a fetal liver library using oligonucleotides based on N-terminal peptide analysis proved that the 120 kd protein is novel. The protein exists in two conformations, the minor one of which more strongly binds to the classical complement pathway component C-4b and, in so doing, inhibits the C3 convertase. The biological function for this interaction remains unknown. Earlier data from the laboratory suggested that fragments of the 120 kd protein are potent vasodilators and, hence, could play a role in mediating certain inflammatory responses. Extensive further tests of that hypothesis could not confirm this activity.

In a second project, Ms. Thelma Gaither continued to explore the effects of oxidative products of neutrophils on membrane receptor function and expression. She had shown previously that hypochlorous acid (HOCl) generated by the neutrophil oxidative burst, reduces the rate of cellular ingestion of opsonized target particles. This year she pursued in vitro studies that demonstrated that physiologic levels of HOCl impair the binding of monoclonal antibodies to the neutrophil surface of Fcγ receptors. Receptor binding was restorable by subsequent treatment with the reducing agents DTT and cysteine, suggesting that, in vivo, an endogenous PMN reductase may be needed to restore Fcγ receptor function that had been impaired by products of the oxidative burst.

Dr. McKenzie has been studying the interactions between complement and viruses. Together with Dr. Murphy (LID), it was shown that complement-mediated neutralization of parainfluenza virus type 3 is dependent upon specific viral envelope binding and destruction by complement components together with antibody. Complement binds alone but antibody directs specific binding of C4 to an epitope of the envelope HN protein, whereupon the full complement cascade is activated and envelope lysis ensues.

Together with Dr. Moss (LVD), Dr. McKenzie characterized further a major protein synthesized by vaccinia. This secreted protein, VCP, is homologous to C4-binding protein. It accelerates the decay of C3 convertase and activates cleavage of both C3b and C4b. These and other findings indicate that VCP may enhance the pathogenicity of vaccinia by inhibiting complement-mediated host defenses.

The **Allergic Disease Section** continued its work over the past year in three major project areas. In studies of nasal physiology, members of the Section defined the causes of rhinorrhea in both allergic rhinitis and upper respiratory tract infections. In both instances, the rhinorrhea is initially due to increased vascular permeability causing the transudation of plasma proteins through the mucosa. During an upper respiratory tract infection the same process is initially responsible for rhinorrhea, but with resolution of infection the dominant origin of secretions is the nasal glands. These observations have implications for pharmacologic management of disease symptoms.

A number of mucous membranes neuropeptides are also under study. The potent bronchoconstrictor peptide, endothelin-1, has been discovered in the nasal mucosa. ET-1 was found in the nasal glands as well endothelial and epithelial cells. RNA message for ET-1 was localized primarily to the endothelial cells, and to a lesser extent to the glands. ET-1 was also proven to act as a mucous secretagogue causing mucous and serous secretion.

ET-1, as well as substance P, neurokinin A, and several other neuropeptides, are degraded by the enzyme neutral endopeptidase (NEP). NEP was localized in the nasal mucosa to serous cell in the submucosal glands and in the endothelium. NEP was secreted along with other glandular products and may degrade neuropeptides found in nasal secretions. The finding of NEP in secretions provide a convenient marker for possible changes in NEP levels as they may relate to degree of nasal airway hyperactivity.

Dr. Hohman has continued studies of the mechanism underlying mast cell mediator release. He has shown that rat basophilic leukemia cell (RBL) become unresponsive to IgE-receptor mediated degranulation after exposure to cyclosporin A or FK-506. Both inhibitors act by binding to immunophilins, cytoplasmic molecules which inhibit PPIase activity. Actions of both cyclosporin A and FK-506 on mast cells occur at concentrations within their pharmacologic range in man, suggesting that both immunomodulators may act by inhibiting mast cell reactions.

Projects being carried out in the **Clinical Mycology Section** are aimed at defining selected aspects of the molecular biology, biochemistry, immunology and treatment of human fungal infections. Dr. Kwon-Chung has a major interest in Cryptococcus neoformans.

During the past year, she identified for the first time the ecological niche of the gattii variety of C. neoformans. She has generated mutants of C. neoformans lacking components of the pyrimidine pathway. Linkage maps based upon these mutants were constructed for various serotypes and varieties of C. neoformans. In other projects, pulse field electrophoresis was used to identify and study chromosomal arrangement in Candida albicans. It was known that diverse strains of C. albicans possess widely differing electrophoretic karyotypes. It was shown that a variety of stresses on C. stellatoidea induce chromosomal alterations. Observations with this strain of Candida will be used to design related studies with pathogenic C. albicans. It is believed that such chromosomal arrangements could be used to find and explain the genetic basis for development of drug resistance by Candida strains.

Dr. Bennet's group has also been interested in basic and biochemical studies of C. neoformans and C. albicans. Dr. Williamson has purified and studied the enzyme kinetics of C. Albicans maltase. Dr. Geber is attempting to clone the C. albican's maltase gene by probing a cDNA library. Initial findings suggest successful identification of a gene that shows extensive homology to the S. carlsbergensis maltase protein.

Dr. Rex has been pursuing investigation of elements of the surface of C. albicans that are capable of binding complement component C3bi. Initial suggest that C. albicans possesses surface proteins that are related to members of the integrin family. The C. albican cDNA library is also being screened to identify genes encoding proteins homologous to mammalian integrins. Ultimately, the group is interested in determining whether the presence of such molecules on the surface of C. albicans affords the organism advantages in terms of virulence and resistance to host defenses.

Members of the Section are directing and participating in a randomized controlled comparison of amphotericin B and fluconazole for treatment of candidemia. Disseminated candidiasis remains a morbid complication of seriously ill, hospitalized patients. The ease of administering fluconazole and its lower toxicity relative to that of amphotericin B, suggest that it may afford equivalent or superior, but preferable, therapy for management of candidemia. This study is projected to end by the end of 1992 and involve 17 study centers.

The **Mast Cell Physiology Section** continues its ongoing study of the clinical features, natural history, diagnosis, and treatment of mastocytosis. During the past year, Dr. Metcalfe helped run a national consensus conference to develop a uniform scheme of classification and evaluation for patients with mastocytosis.

In vitro studies of mast cells have yielded major new insights over the past year. Members of the Section have previously shown that IL-3 outgrowth of mast-like cells from human bone marrow CD34+ progenitor cells. In the past, scientists at Amgen cloned and characterized a new human mast cell growth factor known as the C-kit ligand. In collaboration with those scientist, Dr. Metcalfe's group showed for the first time that this growth factor promotes maturation of cultured mast cells. The availability of C-kit ligand and IL-3 now make it possible to conduct detailed and meaningful analysis of human and animal mast cell growth and differentiation.

In this regard, Dr. Burd has began detailed studies of mast cell gene expression and regulation. One immediate goal is to generate a cDNA subtraction library of genes that are expressed during mast cell activation. This will delineate the cytokines mediators that are synthesized in response to activation signals.

One project continues to address the challenging subject of food allergy. During the past year, control studies involving blinded challenges failed to reveal a consistent pattern of adverse pulmonary reactivity to monosodium glutamate. In collaboration with colleagues in India, efforts are underway to clone and express one very well described allergen from shrimp. The availability of a pure specific allergen of this type should set a new standard immunodiagnosis and desensitization to allergens.

The **Bacterial Pathogenesis Unit** was created this year to conduct studies under the direction of Dr. Russo. Three closely integrated projects are being carried out, each dealing with aspects of the molecular basis of E. coli resistance to host defenses, primarily killing in complement-containing serum. In the initial efforts this group created a large family of E. coli mutants that lose resistance to killing in serum by virtue of transposon sequences inserted into strategic genome sites. The hope is to characterize each of the genes whose deficiency permits killing to generate a comprehensive library of bacterial factors necessary for resistance. In related projects, the group has begun an investigation of the regulation and expression of each of these potential virulence factor. The ultimate goal of the program is to define new targets for disrupting E. coli virulence.

The **Mucosal Immunity Section** has made excellent progress in several of its ongoing research projects. Prior to his departure to become Professor, and Chief of the Division of Gastroenterology at the University of Maryland, Dr. Stephen James developed a means of performing RNA PCR determination so as to quantitate the levels of specific mRNA's. Using this tool, he measured levels of lymphokines in intestinal biopsy specimens with Crohn's disease and ulcerative colitis and compared these with levels in normal tissues and lymphocytes from patients and controls. He found that intestinal lymphocytes synthesize higher levels of INF- γ , IL-2, and IL-5 message than do lymphocytes. Most importantly, Crohn's disease intestines showed markedly elevated IL-2 levels while those of ulcerative colitis patients were normal. This suggests a role for IL-2 in the pathogenesis of the Crohn's form of Inflammatory Bowel Disease. Dr. Strober plans to extend these studies.

Dr. Strober's effort to define the processes governing differentiation of IgA B cell has proceeded. The lab has developed a model system in which to study the differentiation of IgM B cells into IgA-secreting cells. It was shown previously that an intermediate step in differentiation involves the generation of cells that express both IgM and IgA. This past year a variety of stimuli and cytokines were tested to determine their effects on rates of this IgA switch. It was shown that switching occurs only in cell that are precommitted to doing so and that TGF- β and IL-4, and particularly the combination of these two factors, induce switching.

Dr. James completed another series of studies Leu8 molecule that participates in T cell regulatory functions. This year it was shown that Leu8 is present in low levels, or even

absent from lamina propria lymphocytes and that failure to make Leu8 results from the demethylation of the Leu8 gene. A related but larger form of Leu8 was found on the surface of neutrophils. Activation of neutrophils results in release of Leu8. This step may be important in permitting neutrophils to migrate into tissues because Leu8 is an important adhesion molecule that binds neutrophils to endothelial cells surfaces.

Dr. Sneller has been engaged in characterization of sub-populations of patients with common, variable immunodeficiency (CVI) and other primary immunodeficiency disorders. This year he studied and defined a new subset of CVI patients who have elevated levels of circulating CD8+ T cells. When purified from these patients, the CD8+ cell synthesized significantly higher levels of IL-2 and γ IFN than comparable numbers of such cells from CVI patients with normal CD8+.

In related studies, Dr. Sneller characterized the immune features of a child with a marked, diffuse, lymphoproliferative disorder and autoimmunity. He found a greater than hundred fold increase in the proportion of circulating T cells that are both CD4 and CD8-negative. The immune and clinical features defined a new human disease that resembles the model disease that develops spontaneously in *lpr* and *gld* mice. In the coming year, Dr. Sneller hopes to explore lymphokine therapies for some of these primary immunodeficiency disorders.

1990 - 1991
Honors and Awards
Laboratory of Clinical Investigation, NIAID

Stephen E. Straus	Selected to be Chief, Laboratory of Clinical Investigation, Virology editor, <u>Infectious Diseases in Clinical Practice</u> Executive Board, Varicella-Zoster Research Foundation
Warren Strober	Appointed Deputy Director, Intramural Research Program, NIAID Deputy editor, <u>Journal of Immunology</u> Director, American Board of Allergy and Immunology
John E. Bennett	Elected Master, American College of Physicians Co-editor, <u>Principles and Practices of Infectious Diseases</u>
Dean D. Metcalfe	Outstanding Service Medal, USPHS Elected Director, American Board of Allergy and Immunology
John Costa	Marion Merrell Dow-American Academy of Allergy and Immunology Scholar in Allergy Award
Brian Wickes	Huppert Research Award for Graduate Student Excellence, Medical Mycological Society of America

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 0043-26 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology and Chemotherapy of Systemic Mycoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

John Bennett, M.D. Section Head, LCI/CMS/NIAID

Others: John Rex, M.D., Medical Staff Fellow, LCI/CMS/NIAID

Antonia Geber, M.D., Medical Staff Fellow, LCI/CMS/NIAID

Peter Williamson, M.D., Medical Staff Fellow, LCI/CMS/NIAID

Virginia Kan, M.D., Guest Worker

COOPERATING UNITS (if any)

Michael Amantea, Pharm.D., CC, NIH; Thomas McCutchan, Ph.D.,
LPD, NIAID; W. Scott Argraves, Ph.D., Jerome H. Holland Research Laboratories of
the American Red Cross, Rockville, Md.; Drs. John B. Robbins, Rachel Schneerson and
Saryamangala J.N. Devi, LDML, CH, NIH and Pfizer Pharmaceuticals, N.Y.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

5.5

PROFESSIONAL

4

OTHER

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) Both passive and active immunity against experimental cryptococcosis could be induced in mice by a vaccine composed of cryptococcal capsular polysaccharide conjugated to tetanus toxoid. (2) *Candida albicans* maltase has been purified and the gene cloned and sequenced. (3) *Cryptococcus neoformans* diphenol oxidase has been released from the cell wall and partially purified. (4) The small subunit ribosomal RNA of *Blastomyces dermatitidis* has been cloned and sequenced. (5) An integrin like protein has been found on the surface of *C. albicans*.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00045-23 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT - Studies of Interaction of Antibody and Complement on Production of Immune DamagePERSONAL TITLE - Principal Investigator Name of laboratory and institute affiliation

P.I.: Michael Frank, M.D. Chief LCI, NIAID

Others: Carl Hammer, Ph.D. Senior Scientist LCI, NIAID
Tom Waytes, M.D. Medical Staff Fellow LCI, NIAID
Robin McKenzie, M.D. Medical Staff Fellow LCI, NIAID

COOPERATING UNIT'S NAME

Bernard Moss, NIAID

LAB BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.5

PROFESSIONAL

2.5

OTHER

1.0

CHECK APPROPRIATE BOXES

X (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK Use standard form, enter Do not exceed 1000 characters

Studies of the purification, mechanism, of action and sequence of a new plasma protein SgP 120 have continued. Studies have continued on the role of complement in the destruction of parainfluenza virus. The mechanism by which antibody augments complement attack has been under continued study. The studies were broadened this year to include studies of vaccinia virus. Here a C4 binding protein like molecule is released from virally infected cells. The mechanism of action of this protein is under study. It has been shown that it has co-factor activity for C3 and C4 destruction and binds to both C3b and C4b. With the departure of Dr. Michael M. Frank from the National Institutes of Health the major thrust of this project moves elsewhere and the project will be discontinued in the Laboratory of Clinical Investigation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00048-21 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR List other professional personnel below the Principal Investigator (Name, title, laboratory, and institute affiliation)

P.I.: Michael M. Frank, M.D. Chief LCI, NIAID

Others: C. Hammer, Ph.D. Senior Investigator LCI, NIAID

M. Basta, M.D. Visiting Fellow LCI, NIAID

P. Langlois, DScN IRTA

COOPERATING UNITS (if any)

L. Fries, Johns Hopkins School of Public Health, Baltimore, MD

LAB BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN YEARS

4

PROFESSIONAL

3

OTHER

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to explore the mechanism of action of intravenous immunoglobulin in reducing autoimmune damage. During this year it was shown that intravenous immunoglobulin does not reduce the amount of C1 bound to targets. However, it does reduce the amount of C4 and C3 bound to targets equally. Preliminary data suggest that C3 binding is not the primary target of intravenous immunoglobulin but that C4 is more important. The actual mechanism of action of the intravenous immunoglobulin and the structural correlates of the target are under investigation. This year with the departure of Dr. Michael Frank from the National Institutes of Health this project is being terminated. Other members of the program will be fulfilling other duties within the Institute.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 0057-18 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Basic Studies on Pathogenic Fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

K.J. Kwon -Chung, Research Microbiologist, LCI/CMS/NIAID

A. Varma, Microbiologist, LCI/CMS/NIAID

B.L. Wickes, Microbiologist, LCI/CMS/NIAID

COOPERATING UNITS (if any)

University of California, San Francisco, Jeff Edman

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

1

OTHER

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

1) Selection of mutants lacking orotidine-monophosphate-pyrophosphorylase (*ura5*) and orotidine-5'-phosphate decarboxylase (*ura3*) from the two varieties of *Cryptococcus neoformans*; 2) Physical mapping of *C. neoformans* genes; 3) Molecular identification of *C. neoformans* var. *gattii* isolated from *Eucalyptus camaldulensis* in Australia and San Francisco; 4) Stress induced chromosomal rearrangement in *Candida albicans*.

Spontaneous mutants requiring uracil were isolated from both varieties of *C. neoformans* by plating on 5-fluoroorotic acid (5-FOA) medium. Of the 36 strains (18 each from the two varieties), 24 generated (12 each) resistant cells requiring uracil for growth. The uracil requiring mutants of *C. neoformans* var. *gattii* were identified either as *ura3* or *ura5* in equal frequency. The *ura3* isolates were identified by enzyme assay while *ura5* was identified by genetic complementation with a *URA5* gene cloned from *C. neoformans* var. *neoformans*. The uracil requiring mutants of var. *neoformans*, however, were almost always *ura5*. A total of 10 isolated genes were used as probes to construct a linkage map by hybridizing the probes to the chromosomes separated by pulsed-field gel electrophoresis. The linkage map of A and D serotypes of *C. neoformans* were nearly identical but differed from that of *C. neoformans* var. *gattii*. The ecological niche of *C. neoformans* var. *gattii* remained an enigma until 1990 when it was isolated from *Eucalyptus camaldulensis* (red river gum tree) in Australia as well as California. The identity of isolates from the trees were confirmed by using a *C. neoformans* ribosomal DNA probe (Gen-probe) and electrophoretic karyotype.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 0058-17 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis and Chemotherapy of Herpesvirus Infections in Man

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.E. Straus	Senior Investigator	LCI, NIAID	
J. Cohen	Senior Staff Fellow	LCI, NIAID	
R. McKenzie	Medical Officer	LCI, NIAID	B. Savarese, Guest
D. Margolis	Clinical Associate	LCI, NIAID	Research Nurse,
D. Paar	Clinical Associate	LCI, NIAID	LCI, NIAID
J. Meier	Clinical Associate	LCI, NIAID	
P. Krause	Clinical Associate	LCI, NIAID	
J. Dale	Clinical Research Nurse	LCI, NIAID	

COOPERATING UNITS (if any)

J. Rooney, Senior Staff Fellow, LOM, NIDR; A. Notkins, Chief, LOM, NIDR; L. Corey
D. Richman, R. Whitley (ACTGs), K. Smiles (Oclassen, Inc.)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

5.2

PROFESSIONAL:

4.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The goals of the project are to characterize the pathogenesis, natural history and therapy of herpes simplex virus and varicella-zoster virus infections. Our clinical emphasis has been on genital herpes in normal and immune-impaired patients. Analysis of 8 years of suppressive acyclovir therapy in patients with frequently recurring herpes have shown the drug to remain effective, to be well-tolerated, and to not induce drug resistance. We continue to study the ability of ultraviolet light and other physical and chemical agents to induce recurrent herpes simplex infections in humans. In a placebo-controlled trial, topical sunblockers were found to significantly prevent uv induced reactivation of HSV-2 infection. A placebo-controlled trial in patients with frequent, spontaneously recurring oral herpes was completed, showing that acyclovir significantly reduces rates of outbreaks.

Also during this past year we conducted a pilot study of new nucleoside analogs, FIAC and FIAU, for treatment of CMV infections in AIDS patients. Preliminary findings showed no effect on CMV at tolerable doses, but a marked suppression of Hepatitis B virus infection was achieved with FIAU, a finding which will be pursued vigorously.

The major thrust of our laboratory effort in this project has been the analysis of HSV latency in human ganglia. During the past year we cloned and sequenced the latency-associated gene of HSV-2, identified its promoter, characterized the kinetics of its transcription, and analyzed its homology to the corresponding HSV-1 gene. We have begun experiments to determine whether this region of the genome accounts for differences in the rate of recurrences between HSV-1 and 2 in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00154-16 LCI												
PERIOD COVERED October 1, 1990 to September 30, 1991														
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Immediate Events in Hypersensitivity														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Michael A. Kaliner, M.D.</td> <td style="width: 40%;">Head, Allergic Diseases Section</td> <td style="width: 10%;">LCI/NIAID</td> </tr> <tr> <td>Others:</td> <td>Martha V. White, M.D.</td> <td>Senior Staff Fellow</td> <td>LCI/NIAID</td> </tr> <tr> <td></td> <td>Yasushi Igarashi, M.D.</td> <td>Fogarty Visiting Fellow</td> <td>LCI/NIAID</td> </tr> </table>			PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID	Others:	Martha V. White, M.D.	Senior Staff Fellow	LCI/NIAID		Yasushi Igarashi, M.D.	Fogarty Visiting Fellow	LCI/NIAID
PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID											
Others:	Martha V. White, M.D.	Senior Staff Fellow	LCI/NIAID											
	Yasushi Igarashi, M.D.	Fogarty Visiting Fellow	LCI/NIAID											
COOPERATING UNITS (if any) Julie Goff, PhD., Georgetown University (Contract # NO-1-AI-22665)														
LAB/BRANCH Laboratory of Clinical Investigation														
SECTION Allergic Diseases Section														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS 1.4	PROFESSIONAL 1.4	OTHER												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> This broad based project has explored various expressions of allergy over the past 15 years, ranging from anaphylaxis to asthma and rhinitis. This past year, this project focused on aspects of non-immunologic mast cell activation by a histamine releasing factor derived from neutrophils (HRA-N). HRA-N activates mast cells to release histamine, but not to generate eicosanoids such as PGD₂. The release of histamine was determined to be independent of eicosanoid production, as full histamine release can occur in response to IgE receptor mediated activation without any associated eicosanoid production. These data indicate that HRA-N and IgE activate mast cells through independent pathways. The response of mast cells to either IgE receptor mediated activation or HRA-N activation can be inhibited by exposure to corticosteroids, which are potent agents used to treat allergic diseases. A new agent for the treatment of asthma is an anti-allergic compound known as nedocromil. Nedocromil was found to reduce human neutrophil derived HRA-N production, providing an additional mechanism of action for this agent. </p>														

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00249-10 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID

Others: Jo Ann Mican, M.D. Medical Staff Fellow LCI/NIAID

COOPERATING UNITS (if any)

Clinical Pathology Department, NIH Clinical Center (Dr. William Travis)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.75

PROFESSIONAL

0.75

OTHER

0.00

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Adults with mastocytosis reportedly have poor attention span, irritability, difficulty in concentration, forgetfulness, confusion, mood fluctuation, and depression thought to be due to excess histamine. We have assessed 12 children with mastocytosis to determine if similar behaviorial pathology is evident. Results of this study indicate no clear excess of pathology exists in children with mastocytosis. Individual children treated with antihistamines have a nonspecific increase in behaviorial difficulties at rates similar to other medically ill groups. No unique behaviorial pattern implicating histamine overproduction was identified.

We participated in a multi-center consensus conference in 1990 to develop a uniform classification for mastocytosis and uniform evaluation. The results of this conference relied heavily on data obtained at NIH. These consensus recommendations are available and will result in a standard approach to the diagnosis and treatment of this disease.

We have completed a study to characterize liver disease in 40 patients with mastocytosis. All patients had routine liver function tests, liver-spleen scan, and liver biopsy, if indicated. We are in the process of reviewing this data to answer the following questions: 1) What is the prevalence of liver disease in this population? 2) What are the characteristic features of this liver disease? 3) What are the indications for liver biopsy? 4) Is there a relationship between liver disease and mastocytosis disease category?

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00271-10 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification and Characterization of Complement Proteins and Fragments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID

Others: Michael M. Frank, Chief, LCI/NIAID

Thomas L. Leto, Senior Staff Fellow, LCI (BDS)/NIAID

Thomas A. Russo, Senior Staff Fellow, LCI/NIAID

Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID

Gail S. Kerr, Visiting Fellow, LCI/NIAID

Gilda Linton, Medical Technologist, LCI/NIAID

Lois Renfer, Chemist, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS.

PROFESSIONAL.

OTHER.

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00275-10 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

The Effect of PMN Oxidative Product on Membrane Receptor Expression and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.	Thelma A. Gaither	Senior Investigator	LCI, NIAID
Others:	Michael M. Frank	Chief,	LCI, NIAID
	Yannick Pilatte	Guest Researcher	LCI, NIAID
	Evelyn Lin	Chemist (technician)	LCI, NIAID
	Tiffani Durant	Laboratory Worker	LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.75

PROFESSIONAL

2.0

OTHER

0.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☒ (b) Human tissues
 ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The effect of products of the oxidative burst on the function of neutrophil FcγR was studied. We have reported that inhibitors of the oxidative burst, i.e., sodium azide and catalase, enhanced PMN FcγR-mediated phagocytosis of SRBC coated with IgG (E-IgG). Recent studies showed that taurine and methionine (inhibitors of the late-forming oxidant, HOCl and its derivatives) also enhanced phagocytosis. E-IgG rosetting and the membrane expression of 2 different Fcγ's (FcγRII and FcγRIII) were also enhanced by the inhibitors, which in striking contrast, had no effect on the expression or function of the C3b receptor (CR1). This suggested that late-forming products of the oxidative burst down-regulate FcγR but not CR1. Incubation of PMN in HOCl resulted in ~50% loss in E-IgG rosetting and a similar reduction in binding of ¹²⁵I- mAb's against FcγRII, FcγRIII, CR3 (iC3b receptor), and β₂ microglobulin (β₂m), but not that of anti-HLe-1, or anti-CD67, which recognize unrelated PMN membrane antigens. CR1-mediated rosetting and membrane expression were not altered by HOCl. Further incubation of immobilized PMN extracts with HOCl resulted in a significantly greater reduction in anti-FcγIII binding in comparison with anti-CR1 binding. Thus, HOCl or its derivatives alter PMN FcγR's such that they are no longer recognized by their specific ligand, IgG, resulting in the modulation of the function of these important opsonic receptors. It was shown, however, that after incubation for 30 min at 30°C, there was significant recovery in binding of anti-FcγRIII to HOCl-oxidized PMN, and that exposure of HOCl- treated PMN to the reducing agent, cysteine, resulted in significant recovery of FcγR antigenic and functional properties. The ability of PMN to down-regulate and up-regulate receptor function may be of importance in their activity at inflammatory sites

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI00278-10 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Components of the Complement Cascade

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID

Others: Michael M. Frank, Chief, LCI/NIAID

Thomas L. Leto, Senior Staff Fellow, LCI (BDS)/NIAID

Thomas A. Russo, Senior Staff Fellow, LCI/NIAID

Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID

Gail S. Kerr, Visiting Fellow, LCI/NIAID

Gilda Linton, Medical Technologist, LCI/NIAID

Lois Renfor, Chemist, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Project Terminated

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279-10 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID
Others: Joaquim Mullol, M.D. Special Volunteer LCI/NIAID

COOPERATING UNITS (if any)

James H. Shelhamer, M.D., Carolea Logun, M.D., Critical Care Medicine Clinical Center, NIH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.1

PROFESSIONAL

0.1

OTHER

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human and feline bronchial and nasal mucosa have been developed in order to examine the controls of mucus secretion. Using feline airway, PAF and GRP caused mucus secretions, and the isolated eosinophil granule protein, ECP, was even more active. Thus, we are continuing to analyze factors possibly participating in asthma for their actions on mucus secretion.

We also compared our human in vivo challenge system with in vitro responses and confirmed that both models are necessary to explain nasal secretory controls. Alpha adrenergic agonists cause mucus secretion.

Endothelin 1 was localized to glandular and vascular tissues in the nasal mucosa, was found in secretions and, in turn, caused the secretion of mucus in vitro. Moreover, ET-1 caused the nasal mucosa to generate eicosanoids, including HETE's. Inhibitors of the lipoxxygenase pathway reduced ET-1 induced mucus secretion, suggesting that ET-1 in part, acts by causing lipoxxygenase production.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00354 09 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Stephen P. James, M.D., Investigator, Mucosal Immunity Section, LCI, NIAID

Rainer Duchmann, M.D., Special Volunteer, Deutsche Forschungsgemeinschaft

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.25

PROFESSIONAL

1.25

OTHER

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Over the last several years we have examined the hypothesis that the inflammatory bowel diseases, ulcerative colitis and Crohn's disease, are due to an abnormal mucosal immune response to one or more ubiquitous antigens in the mucosal environment. During this period we explored this possibility by measuring lymphokine production by lymphocytes present in mucosal biopsies of patients. In initial studies we developed a quantitative reverse transcription polymerase chain reaction (PCR) method based on the use of varying amounts of "standard" mRNA constructs that are co-amplified with the "unknown" mRNA in specimens; amplification of "unknown" that produces a signal equivalent to "standard" of known initial concentration provides a firm estimate of "unknown" initial concentration. The method was developed in a way that allows standards for four different lymphokines to be added simultaneously and then amplified separately, depending on the primers added. Finally, mRNA transcription was normalized for the number of lymphocytes present in intestinal tissue by quantitating TCR mRNA transcription. We found that IL-2 mRNA transcription in inflamed tissue of patients with Crohn's disease was very considerably elevated as compared to uninfamed tissue and tissue from control individuals. In contrast, IL-2 mRNA transcription from inflamed tissue of patients with ulcerative colitis was normal. Finally, IL-2 mRNA transcription of peripheral blood lymphocytes was low and not significantly different in all groups of patients. These studies establish, therefore, that: 1) Crohn's disease is associated with increased IL-2 production; and 2) Crohn's disease and ulcerative colitis are, from an immunologic point of view, very different disease processes. In other, complementary studies, PCR-based quantitation of IFN- γ , IL-4 and IL-5 in intestinal cells and peripheral blood lymphocytes (PBL) was performed. It was established that IL-2, IFN- γ and IL-5 production by normal intestinal T cells is far higher than in PBL, whereas IL-4 is produced by PBL, but usually not by the intestinal cells. These studies set the stage for quantitative studies of IFN- γ , IL-4 and IL-5 in IBD.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00355 07 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Immunoregulatory defects in primary biliary cirrhosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Stephen P. James, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This project has not been active and we request that it be terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z0A AI 00356-09 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies of the Regulation of IgA Immunoglobulin Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID
Other: Gregory R. Harriman, M.D., Medical Staff Fellow, LCI/NIAID
Rolph Ehrhardt, M.D., Special Volunteer, supported by the
Deutsche Forschungsgemeinschaft

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

3.0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

The overall goal of this project is to gain a cellular and molecular definition of the events governing the differentiation of IgA B cells. During this period we focused on the effects of various cytokines on IgA B cell switching, both in normal spleen B cells and in the B cell line CH12.LX. In addition, we examined the molecular basis of dual-bearing sIgM+/sIgA+ CH12.LX cells. In initial studies, we determined the effect of TGF- β on normal spleen B cells induced by four distinct B cell activation stimuli: 1) LPS; 2) anti- δ -dextran; 3) membrane components of an irradiated, activated T cell clone (D10); and 4) polyclonal cognate interaction. We showed that TGF- β induces small increases in surface IgA expression (up to 2-3% of the cells present) following B cell activation by each of these stimuli, but caused increases in IgA secretion only when cells were activated by LPS. The small amount of switching induced by TGF- β in these various activation systems provides strong evidence that TGF- β is not a primary switch factor; instead, it is an enhancer of IgA switching induced by the activation stimulant itself. In the next series of studies, we determined the effects of TGF- β and IL-4 on IgA switching in the B cell line, CH12.LX. Here, we showed first that CH12.LX cells are pre-committed to switch to IgA since they produce IgA but not IgG subclasses following LPS stimulation, have demethylated I α and C α gene regions and produce germline α mRNA. Next, we showed that TGF- β , IL-4 and particularly the combination of the two, induces 60% of CH12.LX to undergo switch to IgA. The massive switch induced by TGF- β in CH12.LX B cells supports the contention that TGF- β enhances IgA switching only after an initial commitment to IgA switching has been taken. In a final series of studies analyzing dual-bearing sIgM+/sIgA+ CH12.LX cells, we first obtained clones of CH12.LX cells representing the various cellular stages of isotype switching in this cell line; we then showed that: 1) μ mRNA and α mRNA transcripts in dual-bearing CH12.LX clones have identical VDJ regions; and 2) the VDJ gene segment in such clones are juxtaposed to a C μ gene, not a C α gene. Thus, these studies suggest that isotype switching is accompanied by a discrete cellular stage in which an unarranged C H region gives rise to both μ and α mRNA transcripts.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00357 06 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies of the Autologous Mixed Lymphocyte Reaction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

P.I. Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID
Other: Senior Investigator, Stephen P. James, M.D., Mucosal Immunity
Section, LCI/NIAID

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0

PROFESSIONAL

0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This project was not active during this period and we request that
it be terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00397-08 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions of C3b with Immunoglobulin G-Regulation of C3b Function by Antibody

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael M. Frank

Chief

LCI, NIAID

others: Milan Basta

Visting Fellow

LCI, NIAID

COOPERATING UNITS (if any)

Louis F. Fries, John Hopkins School of Public Health

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project has been terminated due to the departure of the Principal Investigator

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 AI 00429-07 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Nasal Responses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Martha V. White, M.D.	Senior Staff Fellow	LCI/NIAID
	David B. Peden, M.D.	Medical Staff Fellow	LCI/NIAID
	Yasushi Igarashi, M.D.	Fogarty Visiting Fellow	LCI/NIAID
	Kimihiko Ohkubo, M.D.	Special Volunteer	LCI/NIAID
	Helen Kaulbach, M.D.	Special Volunteer	LCI/NIAID
	Phyllis Brayton	Microbiologist	LCI/NIAID
	Con't on page two		

COOPERATING UNITS (if any)

James H. Shelhamer, M.D., Critical Care Medicine Clinical Center, NIH
Robert Lebovics, M.D. NIDCD, NIH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The nasal mucosa is the first internal surface to encounter aeroallergens, airborne pathogens, and airborne toxins. Analysis of nasal responses will likely provide insights into normal host defense mechanisms at the mucous membrane level. Allergen challenge results in a direct vascular permeability due to mast cell derived mediators and reflex glandular secretions. An anti-inflammatory agent, nedocromil sodium, had no protective effect on responses caused by allergen. The three divisions of innervation in the nasal mucosa were found to have discrete associations with neuropeptides: The sensory nerves contain GRP (a glandular stimulant), CGRP, SP, and NKA (which act to regulate vasodilation and vascular permeability); the parasympathetic nerves contain VIP (a potent glandular stimulant); and sympathetic nerves contain NPY (a vasoconstrictor). Patients with recurrent sinusitis were found to have a singular defect in nasal secretory responses to cholinergic stimulation. Therefore, these patients may be predisposed to recurrent infections by the absence of specific and nonspecific host defense molecules. Uric acid was found to be the major, stable antioxidant in secretions. Uric acid secretion appeared to be glandular in origin. IL-4 caused histamine release in vivo and increased reactivity to histamine releasing factors in vitro. IL-4 induced nasal congestion may be caused by this increased sensitivity. URI's are universally experienced infections causing rhinorrhea. The source of the rhinorrhea during the initial phase of a URI is the vascular bed due to increased vascular permeability. During the resolution phase of a URI, the source of secretions is glandular. The neuropeptide degrading enzyme, NEP, is found in the nasal mucosa and in secretions. We will use the secretion of NEP to monitor its role in airway reactivity. Nasal urea allows a close estimate of the volume of the epithelial lining fluid, and, calculation of the actual concentration of molecules in secretions. These nasal urea measurements will greatly enhance the analysis of nasal secretions.

Giorgio Piacentini	Special Volunteer	LCI/NIAID
Chul Hee Lee	Special Volunteer	LCI/NIAID
Berhard Mosimann	Special Volunteer	LCI/NIAID

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00430-07 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Biology of Varicella Zoster Virus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J.I. Cohen Senior Staff Fellow
S. Straus Senior Investigator
D. Paar Medical Staff Fellow
J. Meier Medical Staff Fellow

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL

2.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to identify and determine the function of varicella-zoster virus (VZV) genes that are expressed during latency and active infection. We have shown that VZV DNA is present in human trigeminal ganglia recovered at autopsy by using the polymerase chain reaction (PCR). RNA transcripts corresponding to immediate-early and early VZV genes have been detected in human trigeminal ganglia using in situ hybridization and Northern analysis. At present, we are further characterizing VZV RNA transcripts in human trigeminal ganglia by using Northern analysis, by reverse transcription of the RNA followed by PCR amplification, and by construction of a cDNA library from human trigeminal ganglia RNA. We have previously identified VZV proteins that transactivate or transrepress other viral genes. We are constructing cell lines that stably express these VZV proteins and will analyze the effect of these proteins during infection of the cells with VZV or herpes simplex virus. In addition, we are constructing VZV mutants which are deleted for the genes encoding transactivation proteins, a transrepression protein, or viral glycoproteins.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00 432 07 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Regulation of immune responses in humans and non-human primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Stephen P. James, M.D., Investigator, Mucosal Immunity Section, LCI, NIAID
Warren Strober, M.D. Chief, Mucosal Immunity Section, LCI, NIAID
Yohko Murakawa, M.D. Visiting Fellow, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

2.5

PROFESSIONAL

2.0

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

In these studies we are attempting to define the immunologic function of cellular subsets bearing the Leu 8 (LAM-1) molecule. During this period we directed our attention to the function of the Leu 8 molecule on various cell types. In studies with CD4⁺ T cells we showed that cross-linking Leu 8 on the cell surface with solid-phase anti-Leu 8 greatly augments proliferation of CD4⁺ T cells induced by anti-CD3, while having no independent effect on CD4⁺ T cell proliferation alone. These studies suggest that the Leu 8 molecule interacts with the CD3 signal transduction complex. In other studies involving CD4⁺ T cells we showed that Leu 8 expression is down-regulated at both the surface protein level and the mRNA level by cell activation, but this down-regulation is rapidly reversible during culture. Loss of Leu 8 following cell activation, however, does not explain the lack of Leu 8 expression in T cells of the lamina propria since the latter do not regain Leu 8 expression during culture and do not express IL-2 mRNA. Nevertheless, these cells were probably derived from Leu 8⁺ cells because the Leu 8 gene of Leu 8⁺ T cells is partially demethylated, i.e., it was once transcriptionally active. The process causing permanent loss of Leu 8 expression in lamina propria T cells remains unknown. Turning attention to the Leu 8 molecule on neutrophils we showed that most Leu 8 molecules on the surface neutrophils have a higher molecular weight than do Leu 8 molecules on lymphocytes and, concomitantly, Leu 8 mRNA derived from neutrophils is predominantly the higher molecular weight species of the two species present. This suggests that most Leu 8 associated with neutrophils is the membrane anchored form rather than the phosphatidyl-inositol (PI)-linked form and, indeed, very little Leu 8 is cleared from neutrophils with PI-linked proteins (those with paroxysmal nocturnal hemoglobinuria) have a more or less normal complement of Leu 8. Finally, in studies of the Leu 8 molecule on B cells we showed that cross-linking of the Leu 8 molecule with solid phase anti-Leu 8 suppressed SAC-induced Ig secretion of Leu 8 B cells almost completely. This striking effect was obtained without any effect on B cell proliferation, IL-2R, or c-myc mRNA expression. These studies indicate that the indirect down-regulation of B cell differentiation mediated by cross-linking of Leu 8 on T cells is complemented by a direct negative effect by cross-linking of Leu 8 on the B cell itself.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00469-06 LCI

PERIOD COVERED

JULY 1990 - JULY 1991

TITLE OF PROJECT (20 characters or less. Title must fit on one line between the borders.)

COMPLEMENT: STUDIES IN VIRAL INFECTION

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

ROBIN MCKENZIE, M.D., LCI, NIAID

MICHAEL FRANK, M.D., LCI, NIAID

COOPERATING UNITS (if any)

BRIAN MURPHY, M.D., LID, NIAID

BERNARD MOSS, M.D., LVD, NIAID

LAB/BRANCH
LCISECTION
CLINICAL IMMUNOLOGY

INSTITUTE AND LOCATION

NIAID/NIH

TOTAL MAN-YEARS
1.0PROFESSIONAL
1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two projects were undertaken to study the interaction of complement with viruses. The first project utilized a monoclonal antibody which caused complement-mediated neutralization of parainfluenza virus type 3. Since neither this antibody nor complement alone neutralized the virus, they provided a model to investigate the interaction between antibody and complement which was crucial for neutralization. Complement was bound to the virus without antibody, but only with antibody was the virus lysed. Results suggested that the antibody directed C4 binding to the area of the HN protein where further complement deposition caused virolysis.

A second project is the characterization of a major protein secreted by vaccinia virus. This protein, which is homologous to C4-binding protein, inhibits activation of human complement thereby potentiating the pathogenicity of vaccinia virus in the human host.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 A1 00470-06 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Chronic Epstein Barr Virus Infection and Chronic Fatigue Syndrome

PRINCIPAL INVESTIGATOR (List other profession personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	S.E. Straus	Senior Investigator	LCI, NIAID
Other:	J. Dale	Clinical Research Nurse	LCI, NIAID
	B. Savarese	Guest Research Nurse	LCI, NIAID
	E. Benson-Grigg	Guest Research Nurse	LCI, NIAID
	R. McKenzie	Medical Officer	LCI, NIAID
	J. Cohen	Senior Staff Fellow	LCI, NIAID

COOPERATING UNITS (if any)

J. Grafman (LMN, NINCDS), H. Rotbart (Univ. of Colorado), M. Demitrack (Univ of Michigan), R. Herman, M. Kling, P. Gold (NIMH), W. Strober, M. Sneller (LCI, NIAID), Scott Fritz (PRI, FCRF)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

3.0

OTHER

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The goals of this project are to characterize severe chronic infections with Epstein Barr Virus and to characterize multiple aspects of the chronic fatigue syndrome. To date this research project has involved nearly 170 patients. Included are 7 patients who were diagnosed with severe chronic EBV infections on the basis of clinical, historical, molecular and serologic features. We continue to examine immunologic features of patients with severe chronic EBV-associated lymphoproliferation and explore treatments. Interferon proved of little value, but immunosuppressive therapies are being used with good results.

Detailed immunologic, neurologic, endocrinologic and psychologic studies are being conducted on selected patients with chronic fatigue. To date we have no consistent laboratory abnormality that permits a clear diagnosis of the chronic fatigue syndrome, however, we have been pursuing the basis and meaning of the group abnormalities in neuropsychiatric, immune and endocrine systems. During the past year we completed a second set of studies of the pituitary-adrenal responsiveness to corticotropin releasing hormone and ACTH and of neuropeptide and catechol levels in spinal fluid. The findings suggest a novel neuroendocrine defect that may indicate deficient central CRH release. Since CRH induces CNS arousal, these neuroendocrine findings suggest a new mechanism whereby the lethargy of Chronic Fatigue Syndrome patients may be explained. During the past year we completed initial controlled immune studies revealing discrete abnormalities in lymphocyte phenotype and in vitro responsiveness to mitogens in patterns suggesting mild immune activation. In the coming year we will focus heavily of these findings.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00495-05 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biochemical Events in Mast Cell Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Michael A. Kaliner, M.D.	Head, Allergic Diseases Section	LCI/NIAID
Others:	Robert J. Hohman, Ph.D.	Expert	LCI/NIAID
	Thomas Hultsch, M.D.	Special Volunteer	LCI/NIAID
	Prescott Atkinson, M.D.	Medical Staff Fellow	LCI/NIAID
	Raynaldo A. Martin	Biologist	LCI/NIAID

COOPERATING UNITS (if any)

Dr. Sue Goo Rhee, Lab. of Biochem., Sect. on Sig. Transduction, Bethesda, MD
 Dr. Stuart Schreiber, Harvard Univ., Cambridge, MA 02138

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The biochemistry of IgE receptor-mediated activation of mast cells is being studied in two separate but complementary projects which are summarized below.

1) Inhibition of IgE receptor-mediated exocytosis from mast cells by the immunosuppressive drugs cyclosporin A (CsA) and FK506. Immunosuppressive drugs such as CsA and FK506 are considered by many to specifically inhibit T cell functions; however, the same concentration of CsA that inhibits T cell receptor-mediated activation of T cells also inhibits IgE receptor-mediated degranulation of mast cells. These studies have been extended by studying the effect of FK506 and its structural analog, rapamycin, on IgE receptor-mediated degranulation of mast cells. The intracellular target for rapamycin and FK506 (FKBP) is distinct from the intracellular target for CsA (cyclophilin). Like CsA, FK506 inhibits degranulation of mast cells without effecting any other IgE receptor-mediated function; however, FK506 is 100 times more potent than CsA. The IC_{50} for inhibition is 200 nM and 2 nM for CsA and FK506, respectively. Rapamycin and 506BD both compete with FK506 for binding to FKBP. Pretreatment of mast cells with either rapamycin or 506BD prevents the inhibition by FK506. Thus, it appears as though a FKBP-FK506 complex as well as a cyclophilin-CsA complex inhibits receptor-mediated activation of mast cells.

2) Activation of membrane-associated phospholipase C by aggregation of IgE receptors. Currently, little is known concerning the mechanism of activation of PLC and, in fact, it is not even known which of the PLC isozymes present in mast cells is involved in receptor-mediated PI hydrolysis. A major obstacle to these studies is the lack of a cell-free system in which this activation can be studied. We have established a system in which aggregation of IgE receptors results in activation of membrane-associated PLC. Rat basophilic leukemia (RBL) cells are activated by aggregation of the IgE receptors. The PLC activity in membranes isolated from activated cells is increased by 150% above membranes from control cells. This system will be used to identify which PLC isozyme is activated and the mechanism of this activation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00496-05 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Interaction Between the Human Immunodeficiency Virus (HIV) and Herpesvirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: S.E. Straus	Head, Medical Virology Section	LCI, NIAID
D. Margolis	Medical Staff Fellow	LCI, NIAID

COOPERATING UNITS (if any)

J.M. Ostrove (Rockville, MD), A.B. Rabson (UMDNJ, Rutgers, NJ)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.8

PROFESSIONAL:

0.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have been studying the molecular mechanism by which HSV-1 activates HIV RNA transcription. Understanding the system could provide general insights into activation and clinical progression of HIV infection. We had shown previously that HSV stimulates HIV transcription in two distinct ways. Using transient expression assays in Jurkat cells with a CAT reporter we found that the bulk of HSV activation is dependant on the integrity of Spl and NF-kB enhancers upstream of the HIV transcription start site. During the past year we examined an alternative activation mechanism. By gel shift we showed that HSV induces a cellular protein(s) that binds near the HIV transcriptional start site. Competition experiments proved the binding specificity of the protein and uV-cross linking studies identified a novel 45kD protein. HSV-1 rapidly and markedly upregulates levels of this protein. We are currently attempting to clone the protein, termed LBP-2, from a Jurkat cell cDNA library.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00513-04 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Dean D. Metcalfe, M.D.	Head, Mast Cell Physiology Section	LCI/NIAID
Others:	Parris Burd, Ph.D.	Senior Staff Fellow	LCI/NIAID
	Helen Thompson, Ph.D.	Visiting Scientist	LCI/NIAID
	Jo Ann Mican, M.D.	Medical Staff Fellow	LCI/NIAID
	Patrizia Germano, M.D.	Visiting Fellow	LCI/NIAID
	Menachem Rottem, M.D.	Medical Staff Fellow	LCI/NIAID

COOPERATING UNITS (if any)

1. Beth Israel Hospital (Galli)
2. LIR/NIAID (Ehrenreich; Siebenlist)
3. Georgetown University (Goff)
4. Amgen (Zsebo)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

5.50

PROFESSIONAL:

4.50

OTHER

1.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)

Human basophils and mast cells develop from CD34-expressing progenitor cells in the presence of IL-3. Mast cells differentiate to mature phenotypes (as determined by appearance of certain protease isozymes and ultrastructural morphology) when cultured over 3T3 fibroblasts. The ligand of the human c-kit protooncogene (stem cell factor; mast cell growth factor) together with IL-3 enhances the proliferation of all cell lineages including basophils and mast cells and contributes to the maturation of human mast cells. Murine mast cells and also basophils develop from murine bone marrow in the presence of IL-3. However, IL-3 is the only obligatory growth factor for murine mast cells but does not function as a growth factor.

Mast cells are found in diverse anatomical locations and adhere to surfaces coated with laminin or fibronectin. This process is enhanced during IgE-mediated activation. Attachment to laminin depends on a 5 amino acid (IKVAV) sequence, and to fibronectin by the RGD sequence. Murine mast cells also express ICAM-1 on their surface.

FC ϵ RI crosslinking results in a genetic induction of many developmentally important genes including transcription factors and pro-inflammatory and growth factor cytokine genes which are differentially regulated. IL-6 is synthesized in vivo in mouse skin after mast cell degranulation. A neuropeptide (substance P) and thrombin induce pro-inflammatory cytokines in mast cells without histamine release.

Murine mast cells secrete endothelin and also have endothelin receptors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00514-04 LCI

PERIOD COVERED

October 1, 1990 to September 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Diagnosis and Treatment of Adverse Reactions to Foods and Additives

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section LCI/NIAID
Others: Patrizia Germano, M.D. Visiting Fellow LCI/NIAID

COOPERATING UNITS (if any)

Indian Institute of Science, Bangalore, India (P.V. Subba Rao)
Mucosal Immunity Section, LCI/NIAID (Jaffe)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

0.75

PROFESSIONAL

0.75

OTHER

0.00

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

We challenged 13 non asthmatics and 30 asthmatics with monosodium glutamate (MSG). Asthma was classified on the basis of clinical history and steroid use. MSG was administered as a graded single blind oral challenge followed by a placebo-controlled double-blind oral challenge in patients with pulmonary reactions during the single-blind challenge. Medications were withheld prior to study. MSG was administered in capsules containing increasing dosages of MSG at 30 min intervals for a total dose of 7.6 gm. A positive test was defined as a decrease in the FEV₁ of greater than 20%. None of the non asthmatic subjects had any change in pulmonary parameters, and only 1 of 30 asthmatics had a significant drop in FEV₁ (22%). This patient was entered into the double-blind challenge and no decrease in FEV₁ was observed. We concluded that the ingestion of 7.6 gm of MSG over 2 hr or 6.0 gm at a single dose poses no consistent respiratory hazard to normal subjects and to the asthmatic population represented in this study.

Interleukin-5 (IL-5), a lymphokine released by specific subsets of T cells following activation, is thought to be an important factor in eosinophil maturation and function. To determine whether local production of IL-5 by T cells in the mucosa could play a role in diseases characterized by mucosal eosinophilia, we have developed a sensitive and specific semiquantitative assay for IL-5 mRNA transcripts in mucosal biopsies. IL-5 mRNA was detected in gastric endoscopic biopsies from patients with eosinophilic gastroenteritis with multiple food allergies, but not in normal gastric mucosa. These results suggest T cell activation and production of IL-5 may play an important role in eosinophilic gastroenteritis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00548-03 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Prevention of Genital Herpes Simplex Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator) (Name, title, laboratory, and institute affiliation)

PI: S.E. Straus	Senior Investigator	LCI, NIAID
R. McKenzie	Medical Officer	LCI, NIAID
J. Dale	Clinical Research Nurse	LCI, NIAID
B. Savarese	Guest Research Nurse	LCI, NIAID
D. Paar	Medical Staff Fellow	LCI, NIAID
D. Margolis	Medical Staff Fellow	LCI, NIAID
P. Kraus	Medical Staff Fellow	LCI, NIAID
J. Meier	Medical Staff Fellow	LCI, NIAID

COOPERATING UNITS (if any)

R.L. Burke, C. Dekker, (Chiron, Inc., Emeryville, CA)
L. Corey, (University of Washington, Seattle)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

1.5

OTHER

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Having completed a decade of work on antiviral treatment of genital herpes. we turned last year to studies of disease prevention. We began with phase 1 assessment of a new recombinant HSV-2 glycoprotein D vaccine in alum. We recruited 24 adults, some with and without prior HSV-1 and/or 2 infection. Vaccinations of 30ug or 100ug were given at 0, 1, 2, and 12 months and were associated with minimal local or systemic reactions. ELISA titers to gD and gB, neutralizing antibodies to HSV2 and lymphocyte blastogenesis are being tested serially. By 18 months of study we've shown excellent primary immune responses to gD and marked (7-15 fold) boosts of titers in previously infected subjects. On the basis of these excellent responses we enrolled 40 patients with recurrent genital herpes into a placebo-controlled vaccine trial in collaboration with the Univ. of Washington. The goal is to determine whether boosted immunity leads to less frequent recurrences. In the coming year we will complete these studies and begin to explore adjuvants and vaccinations with gB and gD.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00589-02 LCI

PERIOD COVERED

August 1989 to June 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Complement Resistance Determinants in *Escherichia Coli*.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: Thomas A. Russo MDCM Senior Staff Fellow LCI, NIAID

Others: Jane Guenther, B.S. Technician LCI, NIAID

Suzanne Wenderoth, B.S. Technician LCI, NIAID

COOPERATING UNITS (if any)

John Foulds, Ph.D., Senior Investigator, NIDDK, LSD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section / Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.0

PROFESSIONAL

0.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Complement resistance is an important virulence factor in the pathogenesis of bacterial gram negative infections. A more complete understanding of its complement resistance determinants may lead to improved or novel modalities for therapeutic intervention as well as an insight as to the mechanism of complement's action. The experimental approach taken for this project involves transposon mutagenesis of the chromosome and plasmid(s) of a clinical isolate of *Escherichia coli* that is highly complement resistant. We are using a transposon system (TnphoA) designed to mutagenize and identify mutants in outer, periplasmic and cytoplasmic membrane proteins. Mutants are screened for an increased sensitivity to serum and the acquisition of this phenotype will presumably be the result of the disruption of genes that play a role in complement resistance. This method makes no assumptions as to what genes contribute to complement resistance and will hopefully identify previously unrecognized gene products that play a role in this process.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 A1 00590-02 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Primary Immunodeficiency Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Michael C. Sneller, M.D., Senior Staff Fellow, Mucosal Immunity Section,
LCI/NIAID

Other: Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI, NIAID

Jonathan Jaffe, M.D., Clinical Associate, MIS/LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, , NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to determine the role of abnormal lymphokine/cytokine expression in the immunopathogenesis of common variable immunodeficiency (CVI) and other immunodeficiency states. During the present period we studied the immunologic properties of an unusual CD8⁺/HLA-DR⁺ T cell subset that is abnormally expanded in a subgroup of patients with CVI. These CD8⁺ T cells produced significantly less IL-2 and more gamma-interferon than did normal CD8⁺ T cells. In contrast, patient CD8⁺ T cells were found to secrete normal amounts of IL-4, IL-5, and GM-CSF. This abnormal pattern of lymphokine expression was limited to CD8⁺ T cells, as purified CD4⁺ T cells from these patients exhibited a normal pattern of lymphokine expression. In addition, the abnormal CD8⁺ T cell lymphokine secretion pattern was associated with a functional defect: patient T cells were more potent at suppressing Ig production by normal B cells than were CD8⁺ T cell from normal controls. Finally, when purified CD8⁺ T cells from CVI patients with a normal CD4/CD8 ratio were examined, they were found to exhibit normal IL-2 and gamma-interferon production. Thus, the expanded population of CD8⁺ T cells in this subgroup of patients exhibit an unusual pattern of lymphokine secretion that appears to define a distinct subset of patients with CVI.

In related studies we identified a patient with a novel lymphoproliferative disorder of TCR α/β CD4-CD8⁻ T cells. The clinical and immunological features of this case closely resemble the lymphoproliferative/autoimmune disease seen in *lpr* and *gld* mice. The large percentage of TCR α/β CD4-CD8⁻ T cells in this patient's peripheral blood allowed us to isolate these cells and characterize their phenotypic and functional properties. These studies define the clinical and immunological characteristics of a new disease entity.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00595-01 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Purification, Characterization and Function of Proteins of the Complement System.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Carl H. Hammer, Senior Investigator, LCI/NIAID

Others: Michael M. Frank, Chief, LCI/NIAID

Thomas L. Leto, Senior Staff Fellow, LCI (BDS)/NIAID

Thomas A. Russo, Senior Staff Fellow, LCI/NIAID

Ruth M. Jacobs, Medical Staff Fellow, LCI/NIAID

Gail S. Kerr, Visiting Fellow, LCI/NIAID

Gilda Linton, Medical Technologist, LCI/NIAID

Lois Renfer, Chemist, LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

1.3

OTHER

1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

We reported on a unique complement regulatory component sgp120 in J. Biol. Chem. 264:2283-2291, 1989. This plasma protein co-isolates with the second component of human complement (C2) on "C4b"-Sephadex but only at 3-5% yield (sgp120-A). The presence in sgp120 of the O-linked carbohydrate galactose was used to develop an affinity isolation procedure. The capacity of sgp120 to bind to the galactosyl-specific lectin, Jacalin was used in part to purify sgp120-I (non-C4b binding form) containing <10% sgp120-A (subsequently recovered by use of "C4b"-Sephadex). Although the two forms are immunochemically indistinguishable by double diffusion analysis, produce similar fragments on kallikrein digestion and have identical N-terminal amino-acid sequences, by a number of criteria these two forms are distinct and in particular sgp120-A possess most of the described functional activity. Dose-responsive inhibition by sgp120 of the Classical Complement Pathway has been confirmed. Sgp120-A demonstrates up to 10 times the specific inhibitory capacity as found for sgp120-I. Data support inhibition of the C3 convertase, EAC14b2a, by competition with C1 and C2 for a site or sites on bound C4b. Sgp120-A does not compete with C4 for binding to its acceptor on the cell surface. We have fully sequenced the initially identified and isolated clone that produces a fusion protein detectable by monospecific antibody to sgp120. The partial deduced sequence contained the N-terminal peptide sequence derived from a C-terminal 35 kDa fragment derived from sgp120-A. We have also identified and fully sequenced three additional sgp120 clones that, although not full length, contain the 16 amino acid sequence determined by N-terminal analysis of the 25 kDa peptide derived from the 85 kDa peptide by kallikrein digestion of sgp120-A. The deduced amino acid sequences for these sgp120 clones are not present in the current protein database containing 70,000 sequences (January 91) and confirms our report of sgp120 as a new plasma protein.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00607-01 LCI

PERIOD COVERED

New Project Just Approved July 3, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Regulation of Cytokine Gene Expression in Mast Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title laboratory and institute affiliation)

PI: Parris Burd, Ph.D. Senior Staff Fellow

LCI/NIAID

Other: Dean D. Metcalfe, M.D. Head, Mast Cell Physiology Section

LCI/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mast Cell Physiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

PROFESSIONAL

0.1 my

OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Nes project created to study the regulation of cytokine gene expression in mast cells. Work in progress includes the comparative structural analysis of promoter elements required for expression of the TCA3 and MIP-1alpha cytokine genes in mast cells and T-cells. We are also constructing a subtracted mast cell activation library in order to identify and characterize mast cell genes transcribed in response to specific signals. Additional studies are in progress to determine the in vivo effects of mast cell-derived cytokines during IgE-mediated mast cell responses.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00621-01 LCI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Biology of Transformation by Epstein-Barr Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: J. I. Cohen Senior Staff Fellow LCI, NIAID

COOPERATING UNITS (if any)

E. Kieff, Harvard University; C. Sample, St. Judes Children's Hospital;
D. Mosler, Medical Biology Institute

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.75

PROFESSIONAL

0.25

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type Do not exceed the space provided)

Epstein Barr virus nuclear protein 2 (EBNA-2) is essential for B cell transformation by the virus. The goal of this project is to determine the function of EBNA-2 in transformation. Since EBNA-2 is known to transactivate expression of Epstein-Barr virus and B cell genes, we determined whether EBNA-2 could directly activate transcription in vitro. Plasmids containing the DNA binding domain of GAL4 fused to portions of the EBNA-2 gene were cotransfected with a reporter plasmid in B lymphoma cells. A 37 amino acid domain of EBNA-2 activated transcription nearly as strongly as the activating domain of herpes simplex VP16. This domain is essential for B cell transformation by the virus. A 14 amino acid peptide had about 25% of the activity of the larger domain. At present, we are trying to determine (1) what cellular proteins interact with the transcriptional activation domain of EBNA-2, (2) what additional domains of EBNA-2 may interact with cellular proteins to bind viral DNA, and (3) whether other transcriptional activators can substitute for the transforming function of EBNA-2. Additional experiments have been initiated to study the ability of primary B cells transformed by EBV mutants to cause tumors in SCID mice.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00617-01-LCI

PERIOD COVERED

March 1991 to June 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Capsule Synthesis in a Pathogenic *Escherichia coli* Isolate

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: Thomas A. Russo M.D.C.M. Senior Staff Fellow LCI, NIAID

Others: Suzanne Wenderoth, B.S. Technician LCI, NIAID

COOPERATING UNITS (if any)

Susan Gottesman, Ph.D., Senior Investigator, NCI, LMP

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.5

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Escherichia coli is the most common cause of urinary tract infection. Its pathogenicity ranges from uncomplicated urethritis or cystitis to pyelonephritis with sepsis and endotoxemic shock. Capsular polysaccharides are believed to be important virulence determinants in a variety of microorganisms. In *E. coli* capsule has been attributed to play a role in the avoidance of killing by both complement and phagocytic cells. The majority of studies on capsular regulation, however, has been done on the group 1 capsule. Therefore we are undertaking studies in a pathogenic K54 isolate to gain insight on the regulation of group 2 capsule production. A more complete understanding of regulation of capsular synthesis may lead to improved or novel modalities for therapeutic intervention. Our approach in studying this question will be to use a combination of gene disruption and complementation experiments to evaluate the role, if any, of group 1 regulatory elements in the regulation of K54 (group 2) capsule synthesis. Once this is established we will be able to identify any novel regulatory proteins present in this pathogenic isolate.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00618-01-LCI

PERIOD COVERED

September 1990 to June 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Acquisition of an Increased Resistance to Host Defenses In Vivo

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.: Thomas A. Russo, M.D.C.M. Senior Staff Fellow LCI, NIAID

Others: Suzzane Wenderoth, B.S. Technician LCI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology/ Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

0.5

PROFESSIONAL:

0.25

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Microbes are versatile organisms that have the ability to adapt to a myriad of environments in which they must subsist. Pathogenic bacteria, such as *Escherichia coli* are no exception to this rule. Its phenotypic characteristics in in vitro medium such as LB broth are not equivalent to its phenotype when it causes a bacteremia. These changes *in vivo* undoubtedly play an important role in pathogenesis. We are therefore interested in identifying the genes responsible for these *in vivo* phenotypic changes and their mode of induction. Initial studies suggest that the combination of serum plus hemoglobin can induce these gene products. To identify these genes we will use either transcriptional fusions or create a subtraction library. Once the genes of interest are identified their protein products can be elucidated.

LABORATORY OF HOST DEFENSES
1991 Annual Report
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PHS-NIH

Summary Statement

LABORATORY OF HOST DEFENSES

National Institute of Allergy and Infectious Diseases

October 1, 1990 to September 30, 1991

Introduction.

The Laboratory of Host Defenses was created in April, 1991 from the Bacterial Diseases Section of the Laboratory of Clinical Investigation. Its two major tasks are (1) to study the basic mechanisms of protection against microbial infection, particularly infection with bacteria and fungi, and place special emphasis on the study of the physiology and molecular biology of phagocytic cell function in these processes; (2) to investigate the basis of the abnormal processes in patients and to develop new therapeutic strategies for enhancement of host defenses in these patients and in other patients with compromised host deficiencies. The Laboratory employs techniques of clinical research, molecular biology and protein biochemistry.

During its first year, the Laboratory pursued clinical studies in patient cohorts collected at NIH over the past twenty years and in normal volunteers, studied the NADPH oxidase system, and investigated chemoattractant receptors of phagocytic cells.

Clinical Studies.

γ -interferon in Chronic Granulomatous Disease.

A four year project studying the efficacy of γ -interferon gamma in chronic granulomatous disease (CGD) came to completion this year with the demonstration that γ -interferon reduces the incidence of life threatening infections in these patients. These studies provided the basis for the FDA to license this drug for use in CGD and provide the basis for future studies evaluating the potential of γ -interferon in other immunocompromised hosts. (J.I. Gallin, H.L. Malech and E. DeCarlo, LHD, NIAID).

Studies of experimental inflammation in normal subjects established the kinetics of inflammatory mediator appearance at inflammatory sites in man. C5a, LTB₄, and γ -interferon are particularly prominent early (within 3 hours) in inflammation and IL- β , IL-8 and IL-4 are also detected early. Late in inflammation (12-24 hours) IL- β , IL-8, TNF- α , GM-CSF are particularly prevalent. These studies provide the basis for looking for abnormal mediator production in patients with depressed inflammation and for selecting which mediators to

target for development of therapeutics to abort the acute and chronic components of inflammation. (J.I. Gallin and E. DeCarlo, LHD, NIAID and D. Kuhns, PRI-Frederick Cancer Research Facility).

Basic science studies of phagocytic cells.

Utilizing cells from patients with CGD five proteins have been defined that assemble at the plasma membrane of cells to form the NADPH oxidase. The genes for all but one of these proteins have been cloned and sequenced. This past year these proteins have been produced in a baculovirus system in large amounts enabling study of structure and function. Other studies have focused on designing retroviruses vectors for inserting these genes into target cells for gene therapy in CGD. The gene for one of these proteins, p47^{phox}, has been transferred successfully into patient B cells. Concurrently studies are being performed to harvest myeloid stem cells as targets for gene therapy and to develop methods for expanding the stem cell populations for future replacement in CGD subjects. These investigations form the basis for studies exploring gene therapy of CGD. (H.L. Malech, T.L. Leto, D. Rotrosen, P. Murphy and J.I. Gallin).

Studies designed to understand the basis for signal transduction in phagocytic cells have resulted in the definition of a family of chemoattractant receptors. A family of formyl methionine phenyl alanine receptors has been cloned and sequenced as has a low affinity receptor for interleukin-8. The genes for the formyl peptide receptors localize to chromosome 19 whereas the IL-8 receptor gene is on human chromosome 2. These receptors are predicted to form a family of chemoattractant receptors with a secondary structure of a cationic amphipathic alpha helix which likely interacts with a common pertussis toxin sensitive G protein. These studies provide new insights as to the mechanism of the first steps of signal transduction in phagocytic cells. (P. Murphy and H.L. Malech).

Administrative, Organizational, and Other Changes.

The Laboratory has no sections. Dr. Harry L. Malech is the Assistant Chief of the Laboratory.

Honors, Awards, and Scientific Recognition.

Dr. Gallin is currently on the Editorial Board of Cellular Immunology, and Advances in Inflammation Research. He is a Co-editor of the text Inflammation, Basic Principles and Clinical Correlates, with a second edition currently under development. Dr. Gallin is President-elect of the International Immunocompromised Host Society and this past year served as the Chairman, of the Phagocyte Gordon Conference. In FY '91 he gave numerous plenary lectures, he was a visiting professor at several major universities and presented at numerous national and international symposia and seminars.

Dr. Malech is currently the Section Editor of the Journal of Immunologic Methods. He also serves as the Vice-Chairman of the NIAID Clinical Research Protocol Subcommittee. In Fy '91 he presented lectures as invited speaker to John Hopkins-School of Medicine, Genentech Inc. and the Key stone Symposia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-16 LHD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Abnormal Host Defense

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:	Harry L. Malech, M.D.	Assistant Laboratory Chief,	LHD/NIAID
	Richard Kenney, M.D.	Clinical Associate	LHD/NIAID
	Ellen DeCarlo, R.N.	Research Nurse	LHD/NIAID

COOPERATING UNITS (if any)

D Kuhns, PRI-Frederick Cancer Res. Facil.; J Rex, J Bennett and D Margolis, LCI/NIAID; D Alling, DIR/NIAID; P Sponseller, Johns Hopkins Hosp.; R T Wall, Anesthesia/CC.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.0

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of these studies was to study patients with abnormal host defense. In FY '91 studies focused mostly on patients with abnormal phagocytic cell function. Particular emphasis was given to patients with chronic granulomatous disease of childhood (CGD), who are the subject of intense study in the Laboratory of Host Defenses. Some of the clinical observations that were made this year included the following: Demonstration of invasive infection with Sarcinosporon inkin in a patient with CHD; demonstration that normal and abnormal neutrophils can cooperate to damage Aspergillus fumigatus, an observation with important clinical implications for use of white cell transfusions in CGD; demonstration that patients with CGD receiving long term prophylaxis with trimethoprim-sulfamethoxazole have reduced frequency of infection against catalase positive bacteria without increased incidence of fungal infection; completion of long term reviews of skeletal involvement by infection in CGD and anesthesia considerations in CGD; the clinical efficacy of γ -interferon in CGD was demonstrated. We continue to monitor and expand these cohorts of patients as well as patients with leukocyte adhesion deficiency and the syndrome of hyperimmunoglobulin-E and recurrent infections.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00481-06 LHD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Phagocyte NADPH Oxidase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Harry L. Malech, M.D. Assistant Laboratory Chief LHD/NIAID

Others: John I. Gallin, M.D. Laboratory Chief LHD/NIAID

Thomas L. Leto, Ph.D. Senior Staff Fellow LHD/NIAID

Daniel Rotrosen, M.D. Senior Staff Fellow LHD/NIAID

Philip Murphy, M.D. Senior Staff Fellow LHD/NIAID

Julie Katkin, M.D. Special Volunteer LHD/NIAID

Sudhir Sekhsaria, M.D. Medical Staff Fellow LHD/NIAID

COOPERATING UNITS (if any) Richard Kenney, M.D. Clinical Assoc., LHD/NIAID

R. Michael Blaese, NCI; Michael Kleinberg, Dept. Medicine, U. Maryland., Daniel Ambruso, Belle Bonfils Blood Center, Denver, CO; R. Levy, Ben Gurion Univ., Beer Sheva, Israel.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.8

PROFESSIONAL:

3.4

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies the NADPH oxidase involved in human neutrophil and monocyte oxidative responses to inflammation and infection, including: (i) biochemistry of oxidase activation; (ii) gene regulation of NADPH oxidase activity; (iii) NADPH oxidase component gene defects causing Chronic Granulomatous Diseases of Childhood (CGD); (iv) retrovirus expression of oxidase components with in vitro model systems for development of gene therapy of CGD. Cytoplasmic oxidase components p47-phox, p67-phox, NCF-3 and membrane cytochrome b₅₅₈ (CYTO b) subunits p22-phox and gp91-phox interact to form a membrane-bound complex producing superoxide. We show that oxidase activation requires an early reaction corresponding to multiple phosphorylation of p47-phox and translocation to the membrane where it interacts with CYTO b. We identified a carboxyterminus sequence of gp91-phox, arg-gly-val-his-phe-ile-phe, which is critical to oxidase activation. Oligopeptides containing this sequence inhibit oxidase activation. Knowledge of phosphorylation events and critical protein domains involved in oxidase activation may allow development of novel therapies to augment or inhibit inflammation oxidants. We show that regulation of oxidase activity in cultured human monocytes correlates most closely with changing levels of cytoplasmic p47-phox protein. In myeloid cells during differentiation, p67-phox was the last component to appear and was therefore the limiting oxidase factor under these conditions. In other studies, delineation of the structure of the p67-phox gene identified normal allelic restriction-length polymorphisms which could be used to mark the parental origin of p67-phox gene. More recently, we have inserted p47-phox cDNA into a retrovirus vector and used this to restore the missing protein to cultured blood cells from a CGD patient with p47-phox deficiency. In related studies CYTO b subunits have been expressed in a baculovirus expression system and cDNA encoding these subunits have also been inserted into retroviral vectors. These studies represent early steps toward the development of genetic reconstitution therapy for CGD.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00521-04 LHD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Cytokines in Host Defense and Inflammation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
	Ellen DeCarlo, R.N.	Research Nurse	LHD/NIAID

COOPERATING UNITS (if any)

D Kuhns, PRI-Frederick Cancer Res. Facil.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project was to catalogue the kinetics of the appearance of cytokines and other mediators of inflammation in an experimental inflammatory response in man. For this study blisters were raised on the forearms of human subjects using a suction blister device. One hour after the blister was raised the blister fluid was evaluated for cells and mediators. The floor of the blister was scrapped for assessment of cell content. A lucite template containing a 1 ml chamber was placed over the blister floor and then filled with 70% autologous serum and the accumulation of cells and mediators into the blister fluid monitored over time. The data indicated that neutrophils increased in the blister fluid throughout 24 hours of study with less than 5% of the cells being mononuclear cells under these conditions. Interestingly, over the first 5 h the floor of the blister contained about 40% mononuclear cells. Neat blister fluid contained small but significantly greater amounts of C5a, LTB₄, Il-4, Il-8 and γ -interferon compared with simultaneously obtained peripheral blood plasma. These mediators continued to increase with γ -interferon peaking at about 6 hours and C5a, LTB₄ peaking at 8-12 hours and Il-4 and Il-8 peaking at 24 hours. The following mediators were not observed before 8 hours and then continued to increase through 24 hours: TNF- α , Il-1 β , and GM-CSF. Il-1 α and Il-2 were not detected in the blister fluid. Thus, different cytokines appear in this experimental inflammatory focus with different kinetics and the source and regulation of the appearance of these mediators now needs to be studied.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00614-01 LHD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Phagocyte Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas L. Leto, Ph. D.	Senior Staff Fellow	LHD/NIAID
Others:	Harry L. Malech, M.D.	Assistant Chief, LHD	LHD/NIAID
	Michael C. Garrett, B.S.	Biologist	LHD/NIAID
	Cheung H. Kwong, M.D., Ph.D.	Medical Staff Fellow	LHD/NIAID
	Julie Katkin, M.D.	PSTP Fellow	LHD/NIAID
	John I. Gallin, M.D.	Chief, LHD	LHD/NIAID

COOPERATING UNITS (if any)

J. T. Curnutte, Scripps Clinic, LaJolla, CA.

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.1

PROFESSIONAL:

2.7

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects
 ☐ (b) Human tissues
 ☐ (c) Neither
- ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to explore structure-function relationships in important phagocytic cell proteins. Current efforts are focused on the components of the NADPH oxidase, a system comprised of both membrane bound and soluble proteins. Interest in this system lies at two levels: 1) Studies on the the structure and function of this enzyme complex will address the molecular basis of a critical host defense mechanism of phagocytic white blood cells, since the NADPH oxidase is responsible for the generation of superoxide and its potent microbicidal oxygen metabolites. 2) The activation and assembly of the oxidase is thought to involve mechanisms common to other intercellular signal transduction systems relevant in all cells, since two of the oxidase components contain sequence motifs (SH3 domains) exhibiting significant homology to p60-src and other important intracellular proteins. We have recently cloned full length cDNAs encoding the four of oxidase factors (p47-phox, p67-phox, p22-phox, and gp91-phox) affected in Chronic Granulomatous Disease of Childhood (CGD) within a number of expression vectors. Two of these factors (p47-phox and p67-phox) have been purified to homogeneity from a recombinant baculovirus system, allowing detailed biochemical studies previously not feasible with the native proteins. These recombinant factors were used to restore cell-free NADPH oxidase activity of CGD patient cytosolic preparations to nearly normal levels. While the two recombinant proteins together were not sufficient to reconstitute the NADPH oxidase when mixed with membranes, a third soluble component (p28-phox) was identified and purified from crude cytosol based on its ability to complement the activities of recombinant p47-phox and p67-phox in a cell-free oxidase assay system. Finally, the roles of src-like sequence motifs in p67-phox have been explored by deletion mutagenesis and reconstitution using the genetically modified recombinant proteins. These src-like sequences do not appear to be critical for oxidase activity, but are thought to function in cellular localization and activation processes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00615-01 LHD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemoattractant Receptors of Human Phagocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long range goal of this project is to understand the mechanisms by which human blood-derived phagocytic cells localize to and become activated at sites of microbial invasion of the host. The first step toward the achievement of this goal was to establish the molecular structure and functional properties of chemoattractant receptors for human phagocytic cells. To this end, we have cloned cDNAs encoding the N-formyl peptide receptor (FPR) and a low affinity interleukin-8 receptor (IL-8R) from an HL60 neutrophil cDNA library. The functional identity of each receptor was proven by the acquisition of ligand-specific calcium mobilization in Xenopus oocytes that had been microinjected with clone-specific cRNA. A human complementary factor(s) encoded by 3.5 kb transcript(s) was discovered that is required for functional FPR expression but not for IL-8R expression in the oocyte. A third cDNA, designated FPRL1, was isolated that encodes a putative receptor that possesses 69% amino acid identity to FPR but that is not a calcium-mobilizing N-formyl peptide receptor. The IL-8R can be cross-activated by two related cytokines, gro and NAP-2. The low affinity IL-8R is 70% identical to a high affinity rabbit IL-8R. Comparison of the two sequences suggests strongly that the binding site for ligand resides in the amino terminal segment of each receptor. FPR and FPRL1 genes are on human chromosome 19 whereas the IL-8R gene is on human chromosome 2. Sequence comparisons identified all four receptors as members of a peptide chemoattractant receptor gene family. The third cytoplasmic loop of all four receptors as well as the C5a receptor is predicted to assume the secondary structure of a cationic amphipathic alpha helix which likely interacts with a common pertussis toxin sensitive G protein. In the course of our oocyte expression studies, we discovered a novel calcium transporter that is activated by the guanine nucleotide binding protein Gs and is potentiated by lanthanum ion over a narrow concentration range.

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1990 to September 30, 1991

RESEARCH PROGRESS

Programs in the Laboratory of Immunogenetics continue to emphasize the gene families that are important to control of immune function. These investigations concentrate on HLA and human TCR gene complexes. In vivo and in vitro studies concerning the role of retroviruses that exercise deleterious effects on the immune system are in progress using animal models developed in the LIG. Additional studies concern cellular control of immune responses to bacterial antigens. These studies utilize basic methodology in molecular biology, genetics and immunology.

Studies on the Human T-cell Receptor Genes

Inheritance patterns of HLA and T cell receptor (TCR α and TCR β) genes play important roles in a variety of immune processes. The extent of the TCR repertoire was investigated in human families by analysis of TCR α and β specific cDNA libraries prepared from RNA samples derived from PHA-stimulated peripheral T lymphocytes using a technique involving the polymerase chain reaction (PCR). Clones were obtained that correspond to 21 different TCR β and 25 different TCR α variable (V) gene families including 4 V β and 6 V α families that have not been described previously (Robinson). Genetic variation in TCR genes was examined by (i) Southern blot analysis of both conventional and pulsed field gels (PFG) using specific DNA probes to identify restriction fragment length polymorphisms (RFLP); (ii) non-denaturing acrylamide gel electrophoresis to identify single stranded conformational polymorphisms (SSCP) and, (iii) direct sequence comparisons of TCR V region genes. All techniques reveal limited polymorphism in TCR gene segments. A single nucleotide substitution identified in the V β 1 gene resulted in an amino acid substitution located within a hypervariable region, whereas nucleotide substitutions in both V β 12 and V α 22 genes were found to be conservative (Day, Zhao). In contrast to the limited polymorphism observed with individual gene segments, TCR haplotypes are highly polymorphic. There is considerable polymorphism in the combination of markers inherited together in both TCR α and TCR β haplotypes. In addition, variability in TCR gene complexes derives from the insertion or deletion of segments of DNA; two frequently occurring insertion/deletion related polymorphisms were found in the TCR β complex. The locations of the IDRP were determined by the development of an extended map of the TCR β complex showing that one involved a stretch of ~30 kb in the V region and another span ~20 kb near the C region.

Studies of Human Class II MHC Genes and Products

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded by the major histocompatibility complex (MHC). These molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules.

Antigen processing for presentation by class II molecules generally involves endocytosis of antigen into an acidic compartment through which newly synthesized class II molecules are passing on their way to the cell surface. Once at the cell surface, the foreign peptide/MHC class II complex interacts with the T cell receptor and the CD4 molecule expressed on the antigen-specific T cell. The aim of these investigations is to define the function of human class II molecules in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens to CD4-positive T cells.

It has been demonstrated that a cytosolic antigen was endogenously processed in infected cells for class II-mediated presentation. Most interestingly, the processing pathway was different from the one utilized for presentation by class I molecules. This unsuspected presentation of endogenous proteins by class II molecules has important implications on the T cell repertoire selection, on T cell tolerance, and on autoimmunity (Jaraquemada, Malnati, Long).

Several staphylococcal toxins bind to MHC class II molecules and have a strong mitogenic effect on T cells, stimulating entire families of T cells according to the V β chain expressed by the TCR. These staphylococcal toxins, such as SEA, SEE and TSST-1, are representative of a new class of antigens, termed superantigens, that do not require processing for presentation. Using a direct binding assay to normal and mutated class II molecules expressed on transfected cells, it was shown that the toxic shock syndrome toxin (TSST-1) requires both α -helices from the α 1 and β 1 domains of the mouse IA class II Molecule for high affinity binding and that SEA and SEE depend on a critical residue on the β chain α -helix (histidine 81) for binding (Karp, Long).

Mechanism of target cell recognition by natural killer cells

Natural killer (NK) cells have been demonstrated to play a role in the control of viral infection before the establishment of a specific cytolytic T cell response mediated by CD3-positive MHC-restricted T cells. Recently, specific recognition of alloantigens by NK cells was also reported. The aim of this study is to define the mechanism of target cell recognition by NK cells. A large panel of clones was derived from pure CD3-CD56⁺ cells of healthy donors to test their ability to lyse virus-infected autologous cells. Surprisingly, only about half of the clones (41/86) were able to kill HHV6-infected autologous PHA-blasts, while all of them lysed the NK-sensitive cell line K562. A group of clones (17) was further characterized for their ability to recognize autologous or allogeneic infected cells, while another group (15) was analyzed to assess the lysis of cells infected with other viruses (EBV and HSV). The results indicated for the first time that cells infected with different viruses are recognized by different sets of clones. This implies specificity in the recognition by CD3-CD56⁺ lymphocytes and that these cells play a role in the immune response against HHV6 and more generally against viral infection (Malnati, Long).

Infection of Rabbits with the Human Retroviruses HIV-1 and HTLV-I

Studies are in progress to further develop the laboratory rabbit as a model system for infection with the human retrovirus HIV-1, to study the effects of HTLV-I in the rabbit and to assess the synergistic effects on HIV-1 infection of superinfection with HTLV-I.

HIV-1 infection in the rabbit. It was previously observed that a single injection of rabbits with human T cells infected with HIV-1 causes seroconversion within four weeks of injection. In the present study progress of infection was monitored by the polymerase chain reaction (PCR) over a time course ranging from 2 to 50 weeks post-infection. Conserved sequences in the *gag* gene of HIV-1 were targeted for polymerase chain reaction amplification of either DNA or RNA from peripheral blood mononuclear cells (PBMC) and from organs and tissues taken at sacrifice. Viral nucleic acid sequences were detected beginning at 4 weeks post-injection in spleen, lung, bone marrow and lymph nodes of some, but not all, infected rabbits. No viral sequences were detected in control animals injected with uninfected human T-cells. The spleen was the most consistent source of positive reactions and detection of HIV-1 was time dependent. Spleens taken at 2 and 3 weeks post-injection were negative (0 positive of 5 tested), but after 10 weeks 83% (10/12) were positive for HIV-1 *gag* sequences. PBMC were a poor source of virus (3/30) until 36 weeks post-injection; superinfection of HIV-1 infected rabbits with an HTLV-1 infected cell line accelerated the appearance of HIV-1 in PBMC. The present results suggest that PCR detection of HIV-1 in the spleen may provide an endpoint for testing agents directed against HIV-1 infection in the laboratory rabbit. (Truckenmiller).

As in human HIV-1 infection, the brain appears to be a favored target in the HIV-1 infection process in the laboratory rabbit. Virus was detected by PCR analysis of RNA samples isolated from different brain regions or by *in situ* hybridization beginning at approximately 8 weeks post-infection. This corresponds to the time at which virus may be found in the spleen but is considerably sooner than it may be detected in the peripheral blood leukocytes. Although the number and location of regions positive for HIV-1 transcripts showed variation from rabbit to rabbit, all infected animals were positive after 11 weeks. The thalamus was the most frequently affected region; viral sequences were never found in the hippocampus. The consistent finding of brain infection will allow a systematic study of HIV-1 pathogenesis in the CNS and will allow evaluation of prophylactic and therapeutic strategies to block the infection of brain. (Kulaga, Recker).

In vitro studies of HIV-1 infection in the rabbit . It is not known what receptor(s) on rabbit cells bind to HIV-1. Studies to determine the effect of CD4 on infection have shown that soluble human CD4 will block infection of certain cells. Recently a line has been derived by transfection of the cell line 6600-5 with a gene encoding human CD4 (Hague). The transfectants were infected with HIV-1 and showed a substantial increase in parameters associated with HIV-1 infection. This increased level of infection could be blocked by soluble human CD4.

Recent attempts to characterize the rabbit CD4 homolog (RbCD4) to serve as a marker for the T helper subset of rabbit lymphocytes have yielded several cDNA clones from a thymus library. Amplification of RNA samples using PCR primers with sequence based on regions of CD4 conserved among the human, rat and mouse molecules were used to derive probes for this effort. Sequence analysis of a clone for part of the coding region (codons 65 to the 3' terminus) indicates that RbCD4 has 75% identity to human and chimp CD4 and about 68% to that of mouse and rat (Hague). The studies of CD4 involvement in HIV-1 infection in the rabbit will include

construction of a transgenic rabbit expressing a human CD4. This will be carried out in collaboration with TGI Inc. under terms of a CRADA.

It was earlier shown that supernatants taken from rabbit macrophages infected with HIV-1 were negative for reverse transcriptase (RT) activity. In these cells other parameters for infection were positive. The activity giving rise to the RT negativity was shown to be a nuclease that can digest either substrates or products of the assay system (Recker). Attempts are being made to circumvent this activity in order to be able to use RT as a measure of cell infection. In addition to finding this activity in the rabbit macrophage line, it has also been found in the human cell line U937 and in adherent macrophages from both rabbit and human peripheral blood.

In vitro and in vivo effects of HTLV-I on rabbits. HTLV-I is able to infect rabbit cells *in vitro* and *in vivo* producing a lymphoproliferative condition such that cell lines can be readily derived. Significant variation in morphology and growth characteristics of 17 cell lines generated by HTLV-I transformation was observed and eight of these cell lines were studied in detail by a variety of techniques. Two of the 8 lines display features most consistent with T-cells, while another pair is closer to macrophages, 4 of the 8 cannot be readily classified because they have features common to both cell types. Analyses included morphological analysis by light and electron microscopy as well as assays for phagocytosis and immunofluorescence staining to detect expression of markers specific for B-cells, T-cells, and macrophages as well as HTLV-I proteins. Northern blots were used to detect expression of IL2 receptor and CD4 genes, and Southern blots were used to assay for rearrangement of T-cell receptor or immunoglobulin genes and integration of HTLV-I proviral genome. Histochemical analysis was applied to detection of specific and nonspecific esterases, alkaline phosphatase, acid phosphatase, and myeloperoxidase. Two of the lines have multiple integration sites for HTLV-I, while the remaining 6 lines have a single integration site. There is a variation in the ability of different lines to support HIV-I infection; susceptibility to infection was increased in the T-cell like lines. These data support the conclusion that HTLV-I is not solely tropic for T-cells but may also infect other cell. These lines are currently being used to characterize and isolate the interleukin-2 receptor of the rabbit and are being used as targets to study HIV-1 infection in the rabbit. (Sawasdikosol).

Additional studies are underway to test the pathogenic effects of the rabbit HTLV-I lines in autologous as well as in RLA matched and unmatched hosts. It is possible that the ability of HTLV-I to cause disease is related to the cell type that transmits it or to the structure of the viral proteins involved in immune recognition. After characterization, the HTLV-I lines are injected into rabbits and the effects monitored. Certain of the lines have been shown to cause death of the animals after an 8 day period. Lymphocytic infiltrates were found in the liver, lungs and spleens of these rabbits. The sequence of virus present in the lines that cause death will be compared to those in cell lines with less drastic *in vivo* effects (Zhao, Robinson).

Rabbit MHC Genes

The screening of a rabbit genomic library yielded two HLA-DR α related clones. One these was previously shown to contain a functional gene (RLA-DR α) closely related to HLA-DR α . The

second DR related clone, designated DF, was mapped to the rabbit MHC class II region by genetic studies. DF contains contiguous sequences that have significant homology to the RLA-DR α transmembrane coding region (contained in exon 4 of RLA-DR α) and to part of intron 4. These two stretches are reversed in DF when compared to other class II genes, and are flanked on both sides by direct repeats of 130 bp with 85% sequence identity. The presence of the transmembrane sequence without the coding sequences that accompanies in DR α and the observation of the repeats suggests that the DF region played a role in evolution of class II genes. Genomic blots revealed sequences hybridizing to DF in all rabbits tested and in other lagomorph species. The ratio of replacement to silent substitutions in the DF transmembrane region differed significantly from functional class II genes suggesting an absence of recent selective pressure. An additional direct repeat of the C type, similar to that found in cytochrome p450, certain class I MHC genes, TCR β and rabbit uteroglobin, was also present in the DF fragment (Kindt, Marche).

A complete physical map of the rabbit MHC is being sought. Genes have been localized and ordered by pulse field gel electrophoresis (PFGE) in the class II and class III RLA regions. Thus far, the map is similar to that reported for the human HLA complex with few exceptions. Linkage studies as well as PFGE analysis have shown that genes for 21 hydroxylase, complement component C4 and tumor necrosis factor are linked in the rabbit MHC in a manner similar to that found for humans. However, certain genes that are duplicated in humans are present only in a single copy in the rabbit. Some genes recently localized to the MHC such as the heat shock protein 70 genes, are being cloned for the rabbit and probes developed in order to map these genes within the RLA complex (Chouchane).

Recent studies on the major histocompatibility complex (RLA) of the rabbit have developed reproducible methods to type rabbits for MHC genes. Current studies are using animals with known MHC types in order to determine the effect of the RLA antigens on immune responsiveness. Investigations are underway in which cellular responses to certain proteins are being tested using cells from MHC typed rabbits. These studies center about development of immunity to structural and regulatory proteins of the human retrovirus HIV-1. Antibody and cellular immune responses to the envelope protein gp160 and to the regulatory protein nef are being studied (LeGuern). Immunity to these proteins have been elicited by injection with either protein or derived peptides and responses to both protein and peptide are being tested. Rabbits having cellular and humoral responses that neutralize virus will be used for challenge with live HIV-1 in an attempt to determine which of these immunization procedures provide protection against virus infection.

A number of antibodies have been used in an attempt to characterize functionally distinct rabbit lymphoid cell populations, to identify cell surface markers for them and to relate these populations to those that are found in humans. A monoclonal antibody, 93C6, that reacts with activated T cells has been identified and its antigen has been found on spleen and appendix cells but not on thymus. Thymus cells become positive for 93C6 when stimulated with T-cell mitogens. Preliminary results suggest that the antigen of 93C6 may be a component chain of the interleukin-2 receptor (Gordon).

The use of the rabbit infection models with HIV-1 and HTLV-1 may provide an opportunity unavailable in human studies to deduce events in the early pathogenesis of these infections. In

order to understand the pathogenesis of retroviral infection in these animals, normal lymphoid cell populations need to be identified and their distribution and antigen expression defined. Our studies have emphasized the distribution of selected antigens in tissues relevant to retroviral infection via mucosal membranes. A developmental approach with the goal of future studies on maternal retroviral transmission to infants has been taken. Present studies focus on the distribution of cells expressing antigens characteristic of lymphoid cell types in gut associated lymphoid tissues (GALT) of developing rabbits.

CYTOKINES ENHANCE THE EXPRESSION OF SUPPRESSOR T CELL ACTIVITY

Previous studies showed that the transfer of spleen cells from mice primed with Type III pneumococcal polysaccharide (SSS-III) causes a decrease in the magnitude of the antibody response in recipients immunized with SSS-III. Such suppression usually requires the transfer of at least 10^7 primed cells in order to be expressed; only under conditions in which there is enrichment of suppressor T cell (Ts) activity is it possible to transfer suppression with 2×10^6 (or less) primed spleen cells. In recent studies, it was shown that significant suppression could be transferred with 10-100 times fewer primed spleen cells providing such cells are treated with rIL-2 before transfer. Similar numbers of spleen cells treated with rIL-4, rIL-5 or interferon γ likewise were effective. Also, the *in vivo* treatment of mice with anti-IL-2 antibody blocked the development of antigen-specific Ts activity. These findings indicate that Ts are greatly influenced by cytokines which appear to be involved in both their activation and clonal expansion. (Taylor, Baker).

MONOPHOSPHORYL LIPID A (MPL) SELECTIVELY ABOLISHES EXPRESSION OF SUPPRESSOR T CELL (Ts) ACTIVITY

Treatment with bacterial MPL abolishes the expression - but not the induction - of CD8+ Ts activity, without adversely influencing functions mediated by CD4+ amplifier T cells (Ta) and helper T cells (Th). This suggests that Ts, once activated, acquire a cell surface receptor for MPL and/or possess a biochemical pathway that is extremely sensitive to being blocked or inactivated by MPL. The ability of MPL to eliminate the expression of CD8+ Ts activity without altering the expression of CD8+ cytotoxic T cell (Tc) function, suggest that Ts and Tc represent separate lineages of CD8+ T cells that can be differentiated by means of their sensitivity to MPL. (Baker, Taylor, Hraba).

LABORATORY OF IMMUNOGENETICS

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October 1, 1990 to September 30, 1991

HONORS AND AWARDS

In the past year Dr. Kindt was given a citation by the Director, NIAID for receipt of the PHS Superior Service Award. He was invited to present talks at the meeting of the Laboratory of Tumor Biology and at a symposium for the NIH Research Day. In addition, he presented an invited talk at a conference on cooperation between industry and the NIH. Laboratory data were presented in symposia at the FASEB meeting in Atlanta, Georgia, and Dr. Kindt presented seminars on laboratory results at the Institut Pasteur, Paris, Boehringer Ingelheim, Richfield, Connecticut, and at T.S.I. Corporation in Worcester, Massachusetts. Dr. Kindt serves on the study section of the Multiple Sclerosis Society and on the NIH Allergy and Immunology study section. He is an outside reviewer for the Oklahoma Medical Research Foundation and serves as an advisor for students in the medical scholars program for the Howard Hughes Medical Institute. This year Dr. Kindt received the Elliot Osserman Award from the Israel Cancer Research Foundation for service to the research grant review section of this society. Dr. Kindt is a member of the scientific advisory boards of Oncor, Inc., Gaithersburg, Maryland, Southern Biotech Inc, Birmingham, Alabama and Innovir, Inc., New York, New York and has been invited to serve on the scientific advisory board of NeuGenes, Inc., Boston, Massachusetts. In addition, Dr. Kindt served on the nominating committee for the FAES and on the nominating committee for the American Association of Immunologists. Dr. Kindt serves as a deputy editor of the *Journal of Immunology* and is on the board of the *Journal of Experimental Medicine*, and is the North American regional editor for *Research in Immunology*.

Dr. Mary Ann Robinson presented an invited lecture at the Walter Reed Medical Center and presented current laboratory data at the ASHI meeting in November and at the Keystone (Colorado) meeting on Self Reactivity and Its Regulation in February. Dr. Robinson served on the Abstract Awards and Selection Committee for the ASHI meeting and as an Adhoc Reviewer for the United States-Israel Binational Science Foundation and for the NIDDK. Ms. Mary Mitchell who works with Dr. Robinson received an Outstanding Abstract Award at the ASHI meeting in November for the abstract entitled "Genetic Variation in Human T Cell Receptor β Chain Genes Involving the Insertion or Deletion of V Gene Segments."

Dr. Phillip Baker presented lectures and seminars at several universities and medical schools. He is on the Editorial Board of the *ASM News*, serves on the Public Relations Committee of the American Society for Microbiology (ASM) and also serves on the Publications Committee of the International Endotoxin Society (IES). He was invited to represent a paper at a symposium on "Regulation of Immune Responses to Bacterial Polysaccharides" at the Annual Meeting of the ASM in Dallas Texas; was the keynote speaker and convened a session at the International Symposium on Epidemiology, Pathogenesis and Prevention of *Haemophilus influenzae* Disease (Veldhoven, The Netherlands); was a guest lecturer at the Vaccine Department, Statens Seruminstitut, Copenhagen, Denmark; and, organized an International Conference on the "Newer Aspects of the Adjuvant Action of Lipid A and Its Analogs", Airlie, Virginia, where he also chaired a session and presented a major paper. He was also invited to present a paper, as well as to chair a session, at the International symposium on Microbial Infections: The role of Biological Response Modifiers" in Clearwater, Florida.

Dr. Christopher E. Taylor was elected Chairman of the Immunology Division of the American Society for Microbiology (ASM) and was invited to convene a symposium on "New Developments in Immune Responses to Microbes" Cytokines, T cell clones, Superantigens and Autoimmunity" at the Annual Meeting of the ASM in Dallas, Texas. He also presented a paper at that meeting, as well as at the FASEB meeting in Atlanta, Georgia, and presented seminars at several universities. He was invited to present a paper at the 5th International Conference on Immunopharmacology: Role of Biological Response Modifiers" in Clearwater, Florida. Also he participated in the "Introduction to the Biomedical Research Program: of the NIAID, during which he gave a lecture and served on the Selection Committee for Summer Students. He also represented the NIAID at the 5th Annual Symposium on Career Opportunities in Biomedical and Public Health Sciences, in New Orleans, Louisiana.

Dr. Eric Long delivered lectures at the University of Montreal, Montreal, Canada and at the Memorial Sloan Kettering Cancer Center in New York. He co-chaired a workshop at the 75th annual FASEB meeting in Atlanta and at the 1991 NIH Research Day festival. In addition, he was an invited speaker at the 75th FASEB meeting in Atlanta and at the summer FASEB Research Conference on "Lymphocytes and Antibodies" in June 1991.

LABORATORY OF IMMUNOGENETICS
ANNUAL REPORT
October 1, 1990 to September 30, 1991

ADMINISTRATIVE REPORT

In the past year, the Laboratory of Immunogenetics moved from the space on the second floor of Building 4 on the NIH campus to the NIAID Twinbrook II Research Facility in Rockville, Maryland. Upon this move, Dr. Kindt assumed a position of Associate Director, Division of Intramural Research for Twinbrook II planning. Dr. Kindt will continue to serve as Chief, LIG. Dr. Mary Ann Robinson was granted tenure this year and her research unit was joined by Dr. Camilla Day from Rutgers University, and Dr. Tongmao Zhao, American Red Cross. Dr. Karyll Barron will be joining Dr. Robinson's group to carry out studies on human genetics in the position of an IPA and will also have a joint position at Children's Hospital in Washington D. C. Dr. Lotfi Chouchane from the Hopital Cochin in Paris, has joined the Immunogenetics Research Section, Sungae Cho from the Medical College of Virginia and Dr. Mark Simpson, DMV will be joining this Section in August. Dr. M.E. Truckenmiller left the Immunogenetics Research section to join an NIMH laboratory group at St. Elizabeth's hospital. Dr. David Karp left the Molecular Immunology section to assume a position in the rheumatology division of the Dept of Medicine at the University of Texas at Dallas, Dr. Moncef Jendoubi and Luis Rivero departed to begin a section in embryonic stem cell biology in the National Eye Institute. Ms. Merce Marti, a guest researcher, joined the laboratory in the summer of 1991. Dr. Paul Roche from Duke University, Valerie Pinet from Hopital St. Eloi, France and Nicolai Wagtmann from Denmark will be joining the Molecular Immunology Section this coming fall. Dr. Thomas Hraba, a senior scientist from Prague, Czechoslovakia, joined the Microbiology and Immunology Section of the laboratory this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-AI-00143-22 LIG
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic control of the Antibody Response to Microbial Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	P.J. Baker	LIG, NIAID
Head, Microbiology & Immunology Section		
Others:	C.E. Taylor	LIG, NIAID
	T. Hraha	LIG, NIAID
Senior Staff Fellow Visiting Scientist		
COOPERATING UNITS (if any) F.S. Ekwunife, Dept. of Natural Sciences, University of Maryland - Eastern Shore, Princess Anne, MD 21853		
LABORATORY Laboratory of Immunogenetics		
SECTION Microbiology and Immunology		
INSTITUTE AND LOCATION NIAID, NIH Twinbrook II Research Facility, 12441 Parklawn Dr., Rockville, MD 20852		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1	0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)		
<p>Lipopolysaccharide-responsive (LPS^r) and LPS-defective (LPS^d) strains of C3H mice differ greatly in the capacity of bacterial monophosphoryl lipid A (MPL) to inactivate suppressor T cell (Ts) function generated after exposure to Type III pneumococcal polysaccharide (SSS-III). This suggests that the activated Ts of such mice differ with respect to (a) a cell surface receptor required for the binding and subsequent internalization of MPL and/or (b) the presence of a biochemical pathway that is extremely sensitive to inactivation by MPL.</p> <p>The predominant isotype of antibody against <i>Pseudomonas aeruginosa</i> LPS in several strains of inbred mice is IgG₃. Treatment with γ-interferon resulted in an increase in IgG_{2a} antibody, regardless of major histocompatibility complex (MHC) haplotype. Thus the isotypic pattern produced in mice immunized with this antigen is not MHC restricted and is influenced greatly by lymphokines.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00144-27-LIG

PERIOD COVERED
October 1, 1990 - September 30, 1991TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P.J. Baker LIG, NIAID Head, Microbiology & Immunology Section

Others: C.E. Taylor LIG, NIAID Senior Staff Fellow
P.W. Stashak LIG, NIAID Microbiologist

COOPERATING UNITS (if any)

K. Meyers, Ribi ImmunoChem Res Inc., P.O. Box 1409, Hamilton, MT 59840; K.T. Takayama, Mycobacteriology Lab., William S. Middleton Memorial VA Hospital, 2500 Overlook Ter., Madison, WI 53705.

LAB/BRANCH
Laboratory of ImmunogeneticsSECTION
Microbiology and ImmunologyINSTITUTE AND LOCATION
NIAID, NIH, Twinbrook II Res Facility, 12441 Parklawn Dr., Rockville, MD 20852

TOTAL MAN-YEARS

1.7

PROFESSIONAL:

1

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Glycosylated proteins (GP), isolated from the gliding bacterium *Cytophaga johnsonae*, possess several biological and immunological properties usually attributed to the lipopolysaccharides (LPS) or endotoxins of gram-negative bacteria. They are able to (a) activate 70Z/3 pre-B cells to synthesize IgM, (b) induce B cells from both LPS-responsive and LPS-defective strains of C3H mice to synthesize non-antigen-specific polyclonal immunoglobulin, (c) induce macrophages to produce tumor necrosis factor (TNF), and (d) modulate the magnitude of the antibody response in the absence of regulatory T cells. This occurs, despite the fact that GP are free of lipid A as well as 2-keto-3-deoxyoctonate, two components that are common to all preparations of bacterial LPS. The ability of lipid A derived from the nontoxic LPS of *Rhodopseudomonas sphaeroides* to block the ability of toxic LPS - but not GP - to induce TNF by macrophages, suggest that GP and LPS may be acting through different mechanisms and/or cell surface receptors to elicit the effects observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-AI-00145-23 LIG
PERIOD COVERED October 1, 1990 - September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mode of Action of Thymus-derived (T) Suppressor and Amplifier Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: P.J. Baker Others: C.E. Taylor T. Hraba F. Esquivel	LIG, NIAID LIG, NIAID LIG, NIAID LVD, NIAID	Head, Microbiology & Immunology Section Senior Staff Fellow Visiting Scientist Visiting Fellow
COOPERATING UNITS (Name) R.E. Ekins, Department of Cellular Immunology, Walter Reed Army Institute of Research, 9620 Medical Ctr Dr., Rockville, MD 20850; K.T. Takayama, Mycobacteriology Laboratory, William S. Middleton Memorial VA Hospital, 2500 Overlook Terrace, Madison, WI 53705.		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Microbiology and Immunology		
INSTITUTE AND LOCATION NIAID, NIH, Twinbrook II Research Facility, 12441 Parklawn Drive, Rockville, MD 20852		
TOTAL MAN-YEARS: <div style="text-align: right;">1.7</div>	PROFESSIONAL: <div style="text-align: right;">1</div>	OTHER: <div style="text-align: right;">.7</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>The prior <i>in vivo</i> depletion of CD4+ T cells resulted in a significant decrease in the magnitude of the antibody response to several bacterial polysaccharide antigens, not know to require CD4+ helper T cells to initiate antibody production. Such suppression appears to be mediated by the inhibitory effects exerted by remaining CD8+ suppressor T cells (Ts); their effects become dominant in the absence of CD4+ amplifier T cells (Ta) that act in a positive and opposing manner to drive immune B cells to proliferate further in response to antigen. Treatment with various recombinant lymphokines (e.g., IL-2, IL-4, IL-5, but not IL-6) increased the expression of Ts activity <i>in vivo</i>; thus, Ts activity is effected greatly by lymphokines that influence the activation and/or expansion of Ts. Also, Ts, once activated, acquire a cell surface receptor and/or possess a biochemical pathway that is extremely sensitive to being blocked or inactivated by bacterial monophosphoryl lipid A (MPL). Such inactivation appears to be restricted to the expression of Ts activity since treatment with the same or larger amounts of MPL has no adverse effect on the expression of several other T cell functions examined.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00166-14 LIG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Thomas J .Kindt Chief LIG, NIAID

Other: Annie LeGuern Visiting Associate LIG, NIAID
Lotfi Chouchane Visiting Fellow LIG, NIAID

COOPERATING UNITS (if any)

Patrice Marche, Institut Pasteur, Paris; Christian LeGuern, Immunology Branch, NCI; Marc Girard, Pasteur Vaccins, Paris

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

Twinbrook II Facility, NIAID, NIH, 12441 Parklawn Drive, Rockville, MD 20892

TOTAL MAN-YEARS

2.7

PROFESSIONAL:

1.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The present study has two goals related to characterization of rabbit MHC (RLA) antigens. The first is to utilize animals with known RLA types in order to study linkage of immune responses to the MHC and the second is to complete the characterization of the rabbit MHC. In the study on immune responsiveness the rabbit is being examined as a possible model to examine responses against HIV-1 infection and HIV-1 immunization. Peptides and proteins from the virus were used as immunogens and both cellular and humoral responses to these were monitored. Virus neutralizing titers of sera from animals given various immunogens is determined and the immunized are then challenged with human cells infected with HIV-1 and their infection status and immune responses monitored.

In the second aim of this study, a complete physical map of the rabbit MHC is being sought. Genes have been localized and ordered by pulse field gel electrophoresis (PFGE) in the class II and class III RLA regions. Thus far, the map is similar to that reported for the human HLA complex with few exceptions. Linkage studies as well as PFGE analysis have shown that genes for 21 hydroxylase, complement component C4 and tumor necrosis factor are linked in the rabbit MHC in a manner similar to that found for humans. However, certain genes that are duplicated in humans are present only in a single copy in the rabbit. Some genes recently localized to the MHC such as the heat shock protein 70 genes, are being cloned for the rabbit and probes developed in order to map these genes within the RLA complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-AI-00168-14 LIG
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cell Surface Markers of Rabbit Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Thomas J. Kindt	Chief LIG, NIAID
Other:	Mark Gordon Bishop Hague	Staff Fellow IRTA LIG, NIAID LIG, NIAID
COOPERATING UNITS (if any) Jean Claude Weill, Basel Institute, Basel, Switzerland; J. Michael Wilkinson, Royal College of Surgeons, London; Mike Roy, Army Research, Fort Detrick, MD; and Tom Ermak, V.A. Medical Center, San Francisco, CA.		
LAB/BRANCH Laboratory of Immunogenetics		
SECTION Immunogenetics Research Section		
INSTITUTE AND LOCATION Twinbrook II Facility, NIAID, NIH, 12441 Parklawn Drive, Rockville, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Experiments are in progress to characterize distinct rabbit lymphoid cell populations, to identify cell surface markers and patterns of gene expression for them and to relate these populations to cell function. Attempts to characterize a CD4 homolog to serve as a marker for the T helper subset of rabbit lymphocytes have progressed in this period. A gene fragment was amplified from rabbit thymus RNA using primers based on sequences of primate and rodent CD4 molecules. This fragment was used to screen a cDNA library and a clone with appropriate characteristics was found. The data available for codons 65 to the 3' untranslated region reveal over 75% sequence identity to human CD4 and about 68% identity to rodent L3T4 molecules. A search is underway for a full length clone; when this is available it will be expressed in mouse cells and monoclonal Ab against it prepared. In addition, genes encoding other molecules of interest to these studies, such as IL-2 and IL-2r are being cloned from cDNA libraries. Studies of lymphoid cell characterization have concentrated on gut associated lymphoid tissue. These studies are given impetus by the fact that HTLV-I infection is readily passed by milk from infected mothers. A number of antibodies are being tested for reactivity with GALT, thymus, fetal liver and bone marrow in young animals to trace developmental pathways and identify T and B cell precursors in the rabbit lymphoid system.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00170-14-LIG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Eric O. Long Visiting Scientist LIG, NIAID

Other: D. R. Karp Senior Staff Fellow LIG, NIAID

M. Jendoubi Visiting Associate LIG, NIAID

M. Malnati Visiting Fellow LIG, NIAID

J. L. Rivero Visiting Fellow LIG, NIAID

COOPERATING UNITS (if any)

N. Braunstein, College of Physicians and Surgeons, Columbia University, New York; D. Jaraquemada, University of Barcelona, Spain.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

Twinbrook II Facility, NIAID, NIH, 12441 Parklawn Drive, Rockville, MD 20892

TOTAL MAN-YEARS:

4.3

PROFESSIONAL:

2

OTHER:

2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An immune response occurs only if a T lymphocyte has been able to recognize the foreign antigen in association with a self molecule encoded by the major histocompatibility complex (MHC). These molecules expressed on antigen-presenting cells are the polymorphic class II MHC antigens. Most foreign antigens must be processed in order to bind to MHC molecules. Antigen processing for presentation by class II molecules generally involves endocytosis of antigen into an acidic compartment through which newly synthesized class II molecules are passing on their way to the cell surface. Once at the cell surface, the foreign peptide/MHC class II complex interacts with the T cell receptor and the CD4 molecule expressed on the antigen-specific T cell. The aim of this project is to define the function of human class II molecules in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens to CD4-positive T cells. It has been demonstrated that a cytosolic antigen was endogenously processed in infected cells for class II-mediated presentation. Most interestingly, the processing pathway involved was different from the one utilized for presentation by class I molecules. This unsuspected presentation of endogenous proteins by class II molecules has important implications on the T cell repertoire selection, on T cell tolerance, and on autoimmunity. Several staphylococcal toxins bind to class II molecules and have a strong mitogenic effect on T cells, stimulating families of T cells with particular V β chains of the TCR. These toxins are representative of the newly described superantigens, antigens that do not require processing for presentation. Using a direct binding assay with normal and mutated molecules expressed on transfected cells, it was shown that the toxic shock syndrome toxin (TSST-1) required sequences in both α -helices of the α 1 and β 1 domains of the mouse class II IA molecule for binding. In addition, a critical residue (histidine 81) was identified in the α -helix of the β 1 domain of HLA-DR1 molecules that controls binding of superantigens SEA and SEE.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00171-14 LIG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Infection of Rabbits with Human Immunodeficiency Virus 1

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thomas J. Kindt Chief LIG, NIAID

Other: Mark Gordon Staff Fellow LIG, NIAID

David Recker Medical Staff Fellow LIG, NIAID

M.E. Truckenmiller IRTA LIG, NIAID

COOPERATING UNITS (if any)

Thomas Folks, CDC, Atlanta; Alan Lock, Division Vet. Path., NIH; Henrietta Kulaga, Neuropsychiatry Br, NIMH; Shiu Lok Hu, Oncogen Inc., Seattle, WA

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

Twinbrook II Facility, NIAID, NIH, 12441 Parklawn Drive, Rockville, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

5.8

1.8

4.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Laboratory studies have continued on the rabbit model for HIV-1 infection. Studies in the current period were directed toward gaining information concerning endpoints that may be used to monitor infection of rabbits. This is a first step in designing experiments to test the effect of prophylactic and therapeutic strategies to block HIV-1 infection in the infected rabbit. Present data indicate that virus may be detected by PCR reaction in the spleen about 10 weeks following a single injection of HIV-1 infected A3.01 cells. When brains were studied from rabbits given the same single injection, virus was detected as early as seven weeks and after 10 weeks all rabbits were positive for HIV-1 sequences in the brain. Both DNA and RNA PCR techniques were used in this study. In addition, *in situ* hybridization has been used to detect viral RNA sequences in organs from rabbits given a single HIV-1 injection. The peripheral blood mononuclear cells (PBMC) from rabbits, however, are a poor source for viral isolation. Approximately 30 weeks post injection PBMC from a significant percentage of rabbits were HIV positive; before this only sporadic positive results were obtained. This period could be shortened by superinfection with HTLV-I. In this case the presence of virus could be detected as much as eight weeks earlier than in those rabbits given only the HIV-1 injection. In collaborative studies with a group at Oncogen, Inc., rabbit antisera with high known neutralizing HIV-1 titers have been used in passive immunization experiments. Antibody was administered to rabbits that were allotype matched to serum samples and samples taken in order to ascertain the titer on day of infection. These rabbits have been infected and organs are presently being monitored for presence of viral sequences. These studies should provide a means to distinguish between humoral and cellular factors involved in the protection against HIV-1 by the normal immune system.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00180-13 LIG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thomas J. Kindt Chief LIG, NIAID

Other:	Bishop Hague	IRTA	LIG, NIAID
	David R. Recker	Medical Staff Fellow	LIG, NIAID
	Sansana Sawsodikosol	Biologist	LIG, NIAID
	Mary Ann Robinson	Expert	LIG, NIAID
	Tongmao Zhao	Staff Fellow	LIG, NIAID

COOPERATING UNITS (if any)

Frances Gillespie, Transgenic Sciences, Inc., Worcester, MA

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

Twinbrook II Facility, NIAID, NIH, 12441 Parklawn Drive, Rockville, MD 20892

TOTAL MAN-YEARS

3.7

PROFESSIONAL:

2.2

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of transformed rabbit cell lines have concentrated on lines transformed using the human retroviruses HTLV-I and on the use of different rabbit lines as host for infection with HIV-1. A number of lines have been derived by *in vitro* transformation with HTLV-1. Eight of these lines were studied in great detail and characterized according to morphology, cell surface markers and basic functional properties. It was found that several of the lines were distinctly macrophage in character while others were more T-cell like. These lines are being introduced into rabbits either into the autologous hosts or into other MHC matched or unmatched rabbits. Preliminary results suggest that certain of these lines cause leukemia like symptoms and can result in death of the rabbit if sufficient numbers of live cells are introduced. All of the cell lines that were derived may be infected with HIV-1 but there is a large variation in the levels of HIV-1 p24 production. In order to determine whether CD4 is the cell surface receptor for HIV-1 in the rabbit, some of the HTLV-I lines that poorly supported infection with HIV-1 were transfected with human CD4. One of these lines showed a 5 to 6-fold increase in the amount of p24 production in the transfectant over the parent cell line. In addition, it was shown that HIV-1 infection of all of the lines, both the parents and the transfectants could be blocked using soluble human CD4. One line with macrophage characteristics was shown to be positive for HIV-1 infection by p24 production, by electron microscopy and by production of viral proteins but reverse transcriptase activity could not be detected in the assay used. It was found that this cell line produced a factor that interferes with the reverse transcriptase assay. This factor was partially characterized as a nuclease that destroys both substrate and products of the reverse transcriptase reaction. The inhibitor has also been found in normal macrophage cultures of both rabbit and human origin, as well as in some human cell lines such as U937.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00389-08 LIG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genotype Analyses of HLA and TCR Genes in Human Families

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: Mary Ann Robinson Expert LIG, NIAID

Other: Camilla Day Senior Staff Fellow LIG, NIAID
Tongmao Zhao Senior Staff Fellow LIG, NIAID

COOPERATING UNITS (if any)

D. Bernard Amos, Duke University; S. Hauser, Massachusetts General Hospital; P. Concannon, Virginia Mason Institute; Dick Kaslow, NIAID, NIH

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

Twinbrook Facility, NIAID, NIH, 12441 Parklawn Drive, Rockville, MD 20892

TOTAL MAN-YEARS

4.0

PROFESSIONAL

2.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inheritance patterns of HLA and T cell receptor (TCR α and TCR β) genes which are known to play important roles in a variety of immune processes have been analyzed in human families. The extent of the TCR repertoire was investigated by analysis of TCR α and β specific cDNA libraries prepared from RNA samples derived from PHA-stimulated peripheral T lymphocytes using a technique involving the polymerase chain reaction (PCR). Clones were obtained that correspond to 21 different TCR β and 25 different TCR α variable (V) gene families including 4 V β and 6 V α families that have not been described previously. Genetic variation in TCR genes was examined by (i) Southern blot analysis of both conventional and pulsed field gels (PFG) using specific DNA probes to identify restriction fragment length polymorphisms (RFLP); (ii) non-denaturing acrylamide gel electrophoresis to identify single stranded conformational polymorphisms (SSCP) and, (iii) direct sequence comparisons of TCR V region genes. All techniques reveal limited polymorphism in TCR gene segments. A single nucleotide substitution identified in the V β 1 gene resulted in an amino acid substitution located within a hypervariable region, whereas nucleotide substitutions in both V β 12 and V α 22 genes were found to be conservative. In contrast to the limited polymorphism observed with individual gene segments, TCR haplotypes are highly polymorphic. There is considerable polymorphism in the combination of markers inherited together in both TCR α and TCR β haplotypes. In addition, variability in TCR gene complexes derives from the insertion or deletion of segments of DNA; two frequently occurring insertion/deletion related polymorphisms were found in the TCR β complex. The locations of the IDRP were determined by the development of an extended map of the TCR β complex showing that one involved a stretch of ~30 kb in the V region and another span ~20 kb near the C region. Analysis of 40 sibling pairs concordant for the relapsing-remitting form of Multiple Sclerosis (MS) revealed that a gene within the TCR β complex or a closely linked locus influences susceptibility to MS.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AI-00525-04 LIG

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Human Natural Killer Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Eric O. Long Visiting Scientist LIG, NIAID

Other: M. Malnati Visiting Fellow LIG, NIAID

COOPERATING UNITS (if any)

P. Lusso, Lab of Tumor Cell Biology, NCI, NIH; A. and L. Moretta, National Institute for Cancer Research, Genova, Italy.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

Twinbrook II Facility, NIAID, NIH, 12441 Parklawn Drive, Rockville, MD 20892

TOTAL MAN-YEARS

2.3

PROFESSIONAL

1.3

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Natural killer (NK) cells have been demonstrated to play a role in the control of viral infection before the establishment of a specific cytolytic T cell response mediated by CD3-positive MHC-restricted T cells. Recently, specific recognition of alloantigens by NK cells was also reported. Most of the cells displaying natural killer activity belong to the CD3-CD4-CD8-CD16+CD56+ subset of peripheral blood lymphocytes. The aim of this study is to define the mechanism of target cell recognition by NK cells. As a first step, we asked whether specificity could be demonstrated in the recognition of virus-infected cells by NK cells. A large panel of clones was derived from pure CD3-CD56+ cells of healthy donors, to test their ability to lyse virus-infected autologous cells. Surprisingly, only about half of the clones (41/86) were able to kill human Herpes virus 6 (HHV6)-infected autologous PHA-blasts, while all of them lysed the NK-sensitive cell line K562. A group of clones (17) was further characterized for its ability to recognize autologous or allogeneic infected cells, while another group (15) was analyzed to assess the lysis of cells infected with other viruses (EBV and HSV). The results indicated for the first time that cells infected with different viruses are recognized by different sets of clones. This implies specificity in the recognition by CD3-CD56+ lymphocytes. Furthermore, it suggests that NK cells play a role in the immune response against HHV6 and more generally against viral infection.

LABORATORY OF IMMUNOLOGY
1991 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1990 through September 30, 1991

Introduction

The goals of the Laboratory of Immunology (LI) are the elucidation of fundamental mechanisms underlying immunologic responses and the application of the resultant knowledge to problems of autoimmunity, immunodeficiency and related immunologic abnormalities. LI has made rapid progress toward these ends through the application of powerful new technologies that have revolutionized modern biomedical science. These include the techniques of contemporary molecular biology, preparation and use of monoclonal antibodies, preparation of transgenic mice, cloning of normal and transformed lymphocytes and flow cytometric analysis. These methods combined with newer techniques of protein chemistry and cell biology have allowed the solution of many of the major problems that have confronted immunologists and should lead to important advances in efforts to control the normal and disordered immune response.

Expression Folding and Assembly of *In Vitro* Synthesized MHC Class I Molecules

The folding of class I and class II MHC molecules and the role of associated chains in this process is critical to the function of these proteins. An *in vitro* system for expressing class I molecules could provide an extremely useful approach to the study of the folding, assembly and peptide-loading processes. LI scientists have succeeded in *in vitro* translation of RNA's for class I chains in a rabbit reticulocyte lysate/canine pancreatic microsome system. The class I protein is effectively translated, vectorially transported to microsomes and processed. However, it fails to assume its normal folded configuration as judged by failure to bind to a series of conformationally sensitive monoclonal antibodies. By carefully adjusting redox potential, intrachain disulfide bonds form and there is evidence that the $\alpha 3$ domain folds. Expression of conformationally sensitive epitopes of the $\alpha 1/\alpha 2$ domains requires the co-translation of $\beta 2$ -microglobulin and can be further induced if the microsomes containing these translation products are lysed into a solution that contains antigenic peptide. Thus, this model system recapitulates most the critical events in the expression of class I MHC molecules: synthesis, transport to microsomes, folding and formation of intrachain disulfide bonds, binding of $\beta 2$ -microglobulin and competence for peptide binding. Such a system should provide a powerful tool to analyze the molecular events in the process of synthesis, transport, stabilization and peptide loading of MHC molecules. (Ribaldo and Margulies, LI/NIAID).

Peptide Bound to Class II MHC Molecules is Necessary and Sufficient for Attainment of a Stable Conformation

Purified class II major histocompatibility complex (MHC) molecules prepared from cell surfaces are highly resistant to denaturation. They migrate in SDS-PAGE as compact, non-covalently bound dimers. LI scientists have obtained evidence that antigen-derived peptide bound to the class II molecule plays a major role in the attainment and maintenance of this compact stable form. Treatment of purified E α^k E β^k class II molecules with acid resulted in loss of peptide and in transition from the compact dimer state to a floppy dimer or to free monomers. When a peptide that could bind to the E α^k E β^k class II molecule was added while the molecule was in acid, it returned to the compact state upon neutralization. By contrast, addition of peptide after the floppy class II molecule had been returned to neutral pH led to only a very slow attainment of the compact state. These results indicate that peptide plays an important role in determining the structure of class II molecules and that peptide loading is much more efficient at acid than neutral

pH. Consistent with the concept that peptide is critical to the attainment of the compact state, newly synthesized class II MHC molecules which have not yet progressed beyond the medial Golgi and have not yet been loaded with peptide have not attained the highly stable compact form characteristic of mature, peptide-loaded class II molecules. These results indicate that the structural behavior of the class II molecule is highly adapted to the physiological conditions under which it acquires antigenic peptides for presentation to T cells. (Sadegh-Nasseri, Hendrix and Germain, LI/NIAID).

β 2-Microglobulin Controls the Binding of Peptide to Class I MHC Molecules

Using genetically engineered constructs of class I MHC molecules that are soluble rather than membrane bound, LI scientists have devised an assay that allows the measurement of the binding affinity and kinetics for the interaction of peptide with class I molecules. From the measurement of the interaction of a soluble H-2L^d class I molecule with a peptide derived from murine cytomegalovirus (MCMV) in which 25% to 100% of theoretical sites on the class I molecule can be occupied by peptide, a K_D for equilibrium binding of 1.1×10^{-6} M has been obtained. Measurement of kinetic constants by dilution of class I molecules that had bound peptide yielded an unexpectedly rapid "off" rate, inconsistent with the equilibrium constant and the measured association rate. However, the same measurement obtained by adding a large excess of cold peptide, without dilution, yielded a dissociation rate consistent with the equilibrium rates. This effect appears to result from a rapid dissociation of β 2-microglobulin when its free concentration is diminished by diluting the reaction mixture. The resulting complex of class I heavy chain and peptide is unstable, accounting for the rapid dissociation of bound peptide. By contrast, when β 2-microglobulin remains bound to the class I heavy chain, peptide appears to be "locked" in its binding site on the class I heavy chain leading to a very slow dissociation rate. This model has considerable significance for *in vivo* presentation of antigen and emphasizes the potential importance of the concentration of β 2-microglobulin in serum and extracellular fluids for the recognition of target cells by specific T cells. (Boyd and Margulies, LI/NIAID).

These studies find a functional counterpart in the capacity of class I molecules to present antigen to specific T cells. A purified soluble H-2D^d molecule was bound to plastic dishes. Its capacity to be "loaded" with a peptide derived from HIV gp120 (p18) and to present that peptide to p18-specific, H-2D^d-restricted T cells was shown to be determined by the conditions under which loading occurred. In particular, the concentration of β 2-microglobulin present during loading determined the efficacy of the stimulatory activity of the plate-bound H-2D^d. It was essential that β 2-microglobulin and peptide be present together, implying that the dissociation of one element (presumably β 2-microglobulin) determined the binding activity of the complex for the other element. These studies indicate that β 2-microglobulin plays a controlling role in the antigen-presentation function of class I MHC molecules. As noted above, changes in serum concentration of β 2-microglobulin could have substantial importance in the regulation of immune responses (Kozlowski, Boyd and Margulies, LI/NIAID; Takahashi and Berzofsky, MB/NCI).

Engagement of the Vitronectin Receptor is Essential for the Activation of a Set of γ/δ T Cells Expressing a C γ 4, V δ 6 T Cell Receptor

A set of T cell lines prepared from murine dendritic epidermal T cells that express C γ 4, V δ 6 T cell receptors (TCR), a receptor different from that expressed by the predominant γ/δ skin T cell, produced cytokines constitutively as did hybridomas derived from them. Study of one such line, that constitutively produces IL-4, revealed that its ability to do is dependent upon its growth in serum and that the components of serum that are essential for IL-4 production by these cells are RGDS-containing extracellular matrix proteins. The capacity of these cells to produce IL-4 was blocked by an antibody to the vitronectin receptor, implying the extracellular matrix proteins mediated their effect through this receptor. Curiously, IL-4 production was also inhibited by

soluble anti-CD3 antibodies and by clonotypic antibodies specific for the TCR expressed by the IL-4-producing cell line, implying that TCR occupancy as well as engagement of the vitronectin receptor was essential for IL-4 production by these cells. Current work suggests that the antigen recognized by the TCR is likely to be an autoantigen, possibly expressed on the responsive T cell itself. When a set of T cell hybridomas was prepared by fusing neonatal thymocytes with BW5147, all those that spontaneously produced IL-4 required interaction with vitronectin receptor to do so and most expressed a γ/δ TCR very similar to that observed in the index line derived from dendritic epidermal T cells. The finding of autoantigen and extracellular matrix protein-dependent lymphokine production by a set of T cells suggests that this is a frequent characteristic in some T cell compartments and that autoantigen may drive lymphokine production, particularly in situations in which the strength of the signal generated may be markedly enhanced by auxiliary stimulation through the vitronectin receptor. Such cytokine production may be of importance in the initiation of immune responses to a wide variety of antigens and in the initiation of inflammatory responses. The major goal of this project is to identify the ligand that interacts with the TCR of these cells and to evaluate the significance of this stimulation in the control of normal immune responses. (Roberts, Kehn and Shevach, LI/NIAID; Moulder and Coligan, BRB/NIAID).

The AP-1 Element in the IL-2 Receptor is Activated in Normal but not Anergic T Cells

Long term T cell clones of the TH1 type produce IL-2, and several other lymphokines, in response to the recognition of antigen presented by competent antigen presenting cells (APC). However, if APC are inactivated, rather than stimulating IL-2 production by TH1 clones, they induce a state of clonal anergy defined by the failure of those cells to produce IL-2 upon restimulation with antigen and competent APC. LI scientists, in collaboration with scientists from LCMI, have sought a molecular explanation for the failure of these cells to produce IL-2. To do so, they initially examined DNA-binding proteins for many of the defined elements in the IL-2 promoter region. They observed that antigen/APC-stimulated normal and anergic T cells of the AE.7 clone were similar to one another in their expression of such binding factors except for the lack of DNA-binding factors for the AP-1 site of the IL-2 promoter in the anergic AE.7 cells. In order to obtain evidence that such an absence was significant in the transcriptional regulation of the IL-2 gene, they developed an efficient system for transfecting AE.7 cells and examined the activity of multimers of various promoter elements on the transcription of a linked chloramphenicol acetyl transferase (CAT) gene. They observed that multimers of the AP-1 element directed CAT transcription in stimulated AE.7 cells but failed to do so in stimulated anergic AE.7 cells. These results establish that one aspect of clonal anergy is the failure to induce or activate proteins that regulate transcription of the IL-2 gene by action on the AP-1 site in its promoter. Efforts to understand the nature of the event, with particular attention to the status of the two cellular protooncogenes, *c-jun* and *c-fos*, that comprise the AP-1 regulatory complex, are now in progress. This experiments may have great significance for understanding and controlling the process of clonal anergy. In turn, this may provide important insights into the development of strategies to up or down-regulate specific immune responses in clinical situations. (Kang, LI/NIAID and HHMI, and Lenardo, LI/NIAID; Schwartz, LCMI/NIAID).

An Antisense I γ 2b Oligonucleotide Increases the Expression of Germline γ 2b Transcripts, Stimulates B Cell Proliferation and Inhibits Ig Secretion

Immunoglobulin class switching is a complex process. One of the final events in this process is recombination between the switch (S) regions that lie 5' to each of the constant (C) region genes. It has recently been shown that prior to recombinational switching, a transcript appears that is encoded by a genetic element (I) located 5' to the S region of the isotype to be expressed, spliced to the C region gene. Furthermore, these germline transcripts are induced by the action of lymphokines that determine the isotype to which switching will occur. LI scientists have utilized phosphorothioate (S)-oligonucleotides in an effort to determine whether the

I γ 2b/C γ 2b transcript has an important function. When an S-oligonucleotide that was "anti-sense" to a sequence beginning at the most 3' transcription start site in I γ 2b was used in cultures stimulated with LPS or LPS plus interleukin-4 (IL-4), it proved to inhibit secretion of Ig's of all isotypes examined by >90%. Rather than diminishing the steady state level of I γ 2b transcripts, this oligonucleotide caused a 10-20 fold increase in the I γ 2b mRNA. Since I γ 2b mRNA half-life was not effected, it was concluded that the effect of the anti-sense oligonucleotide was at the transcriptional level. The anti-sense oligonucleotide also proved to be a profound stimulant of B cell DNA synthesis and synergized with anti-Ig or LPS in causing even more striking DNA synthesis. Analysis of oligonucleotides in which bases in the index anti-sense sequence were varied indicated that there was a strong correlation between the capacity of an oligonucleotide to hybridize to a ³²P-labelled "sense" oligonucleotide and its biologic activity. In particular, a core sequence in the middle of the anti-sense oligonucleotide appeared essential for biologic activity. It has been proposed that the induction of I γ 2b transcripts as a result of addition of the anti-sense oligonucleotide may mediate the induced biologic functions. These observations may indicate the existence of a previously unrecognized, but exceedingly potent pathway of B cell activation. (Tanaka, Chu and Paul, LI/NIAID).

VEA (Very Early Antigen) is a T Cell Surface Candidate for a Receptor for Accessory Cell Derived Costimulatory Signals

LI scientists have developed a monoclonal antibody (H1.2F3) that recognizes an 85 kD membrane protein not found on resting T cells but rapidly induced upon such cells upon culture in phorbol esters. This antigen appears to homologous to the very early antigen expressed on activated human lymphocytes and designated CD69. F(ab')₂ fragments of H1.2F3 markedly inhibited responses of T cells to stimulation by several accessory cell-dependent stimuli (Con A alloantigen, antigen) but had no effect on responses to accessory cell-independent stimuli such as immobilized anti-CD3. Analysis of the inhibitory activity of H1.2F3 indicated that it was at the level of production of IL-2 since the addition of IL-2 to cultures reversed the inhibitory activity. The inhibition of activation caused by H1.2F3 resembles that observed in the induction of clonal anergy in long term clones of CD4⁺ T cells that have the T_{H1} phenotype. When these cells are stimulated by antigen on fixed antigen-presenting cells, they fail to produce IL-2 and enter a state of anergy characterized by the inability to produce IL-2 in response to stimulation by antigen together with competent APC. Preliminary evidence suggests that stimulation of T cells from primed donors with antigen and competent APC in the presence of (Fab')₂ fragments of H1.2F3 leads to unresponsiveness to that antigen without diminishing the ability of the cell population to respond to other antigens. Efforts are now underway to identify the cell surface ligand, expressed on accessory cells, that binds to VEA. The identification of the receptor for accessory signals and its ligand would be an accomplishment of great significance and would provide new approaches to the manipulation of the immune response either through enhanced induction of unresponsiveness (by blocking VEA or its ligand) or through stimulation of more striking responses (by "hyperstimulation" of VEA). (Dos Reis and Shevach, LI/NIAID).

Interleukin-2 (IL-2) Prepares Activated Mature T Cells to Undergo Apoptosis in Response to Receptor-Mediated Stimulation

IL-2 is principally known for its potent growth promoting activities on T cells and other cell types. LI scientists have now shown that its actions are considerably more complex and that, under certain conditions, it may induce a process leading to the death of T cells through a mechanism characterized by DNA fragmentation, known as apoptosis. Cloned T cells are stimulated to grow by the presence of IL-2, presumably as a result of the binding of IL-2 to high affinity receptors for IL-2 expressed on such cells. These cells may also be stimulated to grow by antigen. However, pretreatment of activated T cell clones with large amounts of IL-2 prior to antigen/APC-stimulation of these cells leads to striking induction of apoptosis. Apoptosis can be

induced in IL-2 treated cloned T cells or in IL-2-pretreated activated normal T cells by mitogens or by the use of calcium ionophores and phorbol esters. Furthermore, evidence from the effect of administering superantigens, such as staphylococcal enterotoxins, multiple times to normal mice indicates that cells that initially respond to the superantigens, defined by their expression of characteristic V β genes, undergo cell death *in vivo*. This superantigen-induced cell death can be blocked by antibodies to the α chain of the IL-2 receptor, supporting the conclusion that IL-2 can sensitize cells for apoptosis *in vivo* as well as *in vitro*. This stimulation-induced cell death may be a major mechanism for the regulation of cell expansion and maintenance of cellular homeostasis in *in vivo* immune responses. (Lenardo, LI/NIAID).

Cells of the Basophil Lineage are Major Producers of IL-4

LI scientists have demonstrated that both transformed and non-transformed long term mast cell lines produce IL-4 and other lymphokines upon engagement of their high affinity receptor for IgE (Fc ϵ RI) or by treatment with ionomycin. Lymphokine production by such cells in response to Fc ϵ RI-cross-linkage is markedly enhanced by pretreatment with interleukin-3 (IL-3). Recently, it has been shown that Fc ϵ RI⁺ cells in the spleen and bone marrow of normal mice are potent-producers of IL-4 in response to cross-linkage of Fc ϵ RI or of Fc γ RIII or to treatment with ionomycin. Indeed, on a per cell basis, Fc ϵ RI⁺ cells from normal or parasite-infected mice are 100-300-fold better than long term mast cell lines in the production of IL-4. Purification of Fc ϵ RI⁺ spleen or bone marrow cells by cell sorting revealed that a substantial portion of these cells could be identified as basophils or basophilic myelocytes based on granule and nuclear morphology. In order to determine the relative contributions of mast cell- and basophil-lineage cells to the IL-4-producing capacity of freshly harvested Fc ϵ RI⁺ cells, bone marrow cells were cultured for 5-7 days in IL-3 or in IL-3 plus "steel factor". The resulting cell population was markedly enriched in Fc ϵ RI⁺ cells which could be subdivided into cells that expressed the protooncogene *c-kit* and those that lacked it. The *c-kit*⁺, Fc ϵ RI⁺ cells had the morphology of mast cells while the *c-kit*⁻, Fc ϵ RI⁺ cells appeared markedly enriched in basophils and basophil lineage cells. The *c-kit*⁺, Fc ϵ RI⁺ cell population were excellent producers of IL-4 in response to cross-linkage of Fc ϵ RI or Fc γ RIII or to ionomycin while the *c-kit*⁻, Fc ϵ RI⁺ cells were much poorer IL-4 producers. These results indicate that basophils or their progenitors have the capacity to produce IL-4 and raise the possibility that they are major contributors to IL-4 production in physiologic and pathophysiologic conditions. (Seder and Paul, LI/NIAID; Plaut, Johns Hopkins; Dvorak and Galli, Harvard Medical School).

Extracellular ATP May Mediate T Cell Cytotoxicity

A major effector function of T lymphocytes is the destruction of specific target cells, such as virally-infected or tumor cells. The mechanisms through which "killer" T cells destroy their target cells has been a subject of great interest and has substantial clinical significance. One major theory is that the granules of cytotoxic T cells contain lytic substances that are secreted by the killer cell when it is specifically stimulated as a result of T cell receptor-mediated recognition of antigens on the target cell. Indeed, cultured cytotoxic T cells contain granules and among the contents of these granules is perforin. The latter is a C9-like molecule that, when polymerized in the membrane of target cells, is capable of lysing the cells. However, studies by LI scientists and others have demonstrated that secretion of perforin by cytotoxic T cells as a result of receptor-mediated activation cannot explain all forms of cytotoxicity since cytotoxic T cell clones can destroy their target cells in the absence of extracellular calcium which is an absolute requirement for stimulation of granule exocytosis. LI scientists have recently observed that substantial amounts of extracellular ATP are produced by cytotoxic T cells as a result of receptor cross-linkage. The appearance of extracellular ATP is calcium-independent and extracellular ATP is a powerful killer of many cells. Furthermore, killer T cells are insensitive to the lytic activity of extracellular ATP. This lack of sensitivity to the putative cytotoxic effector can be explained by the fact that these cells

express large amounts of ecto-ATPase, capable of degrading ATP. When cells are lysed by extracellular ATP, they display DNA fragmentation similar to that observed when target cells are killed by cytotoxic T cells; perforin-mediated killing does not result in DNA fragmentation, reaffirming the concept that it cannot account for all aspects of T cell-mediated cytotoxicity. Studies of pH and Mg^{2+} -dependence of CTL-induced cytotoxicity suggest that the active form of ATP is MgATP rather than ATP^{4-} . Direct evidence for the role of ATP in cell killing is now being actively sought. The analysis of this novel mechanism of cytotoxicity can address the precise cellular and molecular events in the killing process and may give insight the details of cytotoxicity. (Filippini and Sitkovsky, LI/NIAID).

Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play important roles in U.S. and International immunological communities. They serve on editorial boards of many scholarly publications. Dr. Ethan Shevach is editor-in-chief of the Journal of Immunology and is a member of the editorial boards of Cellular Immunology, the Journal of Immunological Methods and of Current Protocols in Immunology. He is a member of the Council of Biology Editors.

Dr. William Paul is the editor of the Annual Review of Immunology. He is an advisory editor of the Journal of Experimental Medicine, an associate editor of Cell, and a member of the editorial boards of Immunological Reviews, of the Journal of Molecular and Cellular Immunology, of Cell Regulation and of Cytokine. He is a transmitting editor for International Immunology and a corresponding editor of the Proceedings of the Royal Society, Series B. He is a member of the editorial advisory board of Advances in the Regulation of Cell Growth. He edited a volume entitled Immunology: Recognition and Regulation, published by H. Freeman, which appeared this year.

Dr. Ronald Germain is deputy editor of the Journal of Immunology. Dr. Rose Mage is a member of the editorial board of Immunogenetics. Dr. David Margulies is an Associate Editor of the Journal of Immunology and an editor of Current Protocols in Immunology. Dr. Michael Lenardo is an associate editor of Molecular and Cellular Biology. Dr. John Inman is a member of the editorial board of Analytical Biochemistry and is an advisory editor of Molecular Immunology.

Dr. Paul chaired the Board of Scientific Consultants of the Memorial-Sloan Kettering Cancer Center and the Advisory Committee of the Harold C. Simmons Arthritis Center at the University of Texas Southwestern Medical Center. He is a member of the Scientific Advisory Board of the Howard Hughes Medical Institute; of the Committee to Visit the Division of Medical Sciences of the Board of Overseers of Harvard College; of the Advisory Committee of the Pew Scholars Program in Biomedical Sciences; of the Fellowship Committee of the Cancer Research Institute, Inc.; and of the Science Board of the Biomedical Research Centre, Vancouver, B.C. He was a co-organizer of the Cancer Research Institute Workshop on Molecular Aspects of B Cell Development and Activation and is co-chairman of the Program Committee for the 1992 Mid-Winter Conference of Immunologists in Asilomar California. He is the Secretary-General of the Ninth International Congress of Immunology, to be held in San Francisco in 1995.

Dr. Paul received an honorary Doctor of Sciences degree from the State University of New York in June of 1991. During the past year, he presented the Anderson Medical Lectures at the University of Virginia School of Medicine, the Virginia Mason Research Institute Distinguished Lecture, the La Jolla Science Lecture and the Wellcome Lectures at Wayne State University School of Medicine. He was the keynote speaker at the Keystone Symposium on Cytokines and their Receptors. In addition, Dr. Paul was a session chairman and a speaker at the Gordon Conference on Immunochemistry and Immunobiology. He was an invited lecturer at the GM Cancer Symposium, at the Nature Symposium on Immunology; at the International Conference on Mast cells in Hiroshima, Japan; at the International Congress of Biochemistry in Jerusalem, Israel; and at the annual meeting of the British Society of Allergy and Immunology.

Dr. Germain is a member of the Scientific Advisory Board of the Ruggero Ceppellini Advanced School of Immunology, in Naples, Italy. He initiated and organized the NIAID Research Grand Rounds. He was the co-chairman of, and an invited speaker at, the FASEB Symposium on Antigen Processing. He was a session chairman and an invited speaker at the Gordon Conference on Immunochemistry and Immunobiology. He was an invited speaker at the Cancer Research Institute Conference on Molecular and Cellular Biology of Antigen Processing

and Presentation and at the NIAID Conference on The Molecular Immunology of Sexually Transmitted Diseases. He was an instructor in the American Association of Immunologists course on Regulatory Mechanisms in Immunity.

Dr. Shevach is a member of the Scientific Advisory Board of the American Leprosy Foundation. He was a session chairman and an invited speaker at the New York Academy of Sciences Conference on T Cell Activation; and at the Conference on Cytokines and Infectious Diseases, held at NIH. He chaired a workshop at the annual NIH Research Day and was a participant at the Workshop on Scientific Integrity for editors of major biomedical research journals.

Dr. Margulies received a USPHS Outstanding Service Medal and was promoted to the rank of Medical Director (O6) in the USPHS Commissioned Corps. He is a member of the Immunology and Immunotherapy Advisory Committee of the American Cancer Society and is a member of the Program Committee of the Howard Hughes Medical Institute Cloister Scholars Program.

Dr. Mage is a member of the Board of Directors of the Foundation for Advanced Education in the Sciences. She delivered the opening lecture at a symposium in honor of Dr. Andrew Kelus at the Basel Institute for Immunology.

Dr. Lenardo was an invited speaker at the Dedicatory Symposium of the Hanson Centre for Cancer research at the Royal Adelaide Hospital in Adelaide, Australia; was an invited speaker at the Gordon Conference on Immunochemistry and Immunobiology; and was a lecturer in the Molecular Biology of Gene Regulation Course at Stanford University.

In addition, Laboratory of Immunology Scientists presented research seminars at major universities and research institutes in the United States and abroad.

Administrative, Organizational and Other Changes

Dr. Michail Sitkovsky was advanced to tenure status in the Laboratory of Immunology. The leadership of the intramural research program and the Board of Scientific Counselors have recommended that a Section on Biochemistry and Immunopharmacology be established in Laboratory of Immunology which he would head.

The Laboratory of immunology continues to be a major training center for young immunologists. During the past year several individuals completed pre- or post-doctoral training periods or sabbatical periods in the Laboratory. Among these were Wolf-Henning Boehnke, John Classen, George Dos Reis, Dan Eilat, Antonio Filippini, Mariagrazia Grilli, Zvi Grossman, Sang-Mo Kang, John Meligeni, Sei-Ichiro Minato, Gib Otten, Geraldo Pereira , Jeffrey Sherman, Rolf Taffs and Deborah Weinstein. Each of these scientists made important contributions to the Laboratory of Immunology research program. It is anticipated that they will have very productive research careers.

During the past year, several scientists joined the Laboratory of Immunology as students, post-doctoral trainees and sabbatical visitors. They include David Agus, Elizabeth Bonney, Flora Castellino, Hua-Tang Chen, David Chin, Lin-Na Ding, Matthew Fitts, Patrizia Fuschiotti, Richard Gala, Melissa Gregory, Joost van Meerwijk, Martin Rocken, Paola Romagnoli and Jeffrey Thomas. It is expected that they will continue a tradition of excellence established by a long series of outstanding trainees in the Laboratory.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Paul	Chief	LI, NIAID
Others:	T. Tanaka	Visiting Fellow	LI, NIAID
	Z.-S. Ye	IRTA Fellow	LI, NIAID
	C. Chu	Guest Researcher	LI, NIAID
	J. Thomas	Visiting Fellow	LI, NIAID

COOPERATING UNITS (if any)

USUHS (F. Finkelman); USDA (J. Urban); CBER, FDA (E. Max)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

3.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunoglobulin class switching is regulated by the action of lymphokines. Of these the best studied example is IL-4 regulation of production of both IgG1 and IgE in vitro and of IgE in vivo. IgE production in both primary and secondary responses to conventional antigens and to infections with parasitic nematodes are inhibited by treatment with antibodies to IL-4 or to the IL-4 receptor. Indeed, in mice infected with *Heligmosomoides polygyrus*, anti-IL-4 receptor antibody not only blocks IgE responses but also blocks the acquisition of protective immunity.

In order to study the process of Ig class switching more precisely, an assay for the DNA deletional event that is involved in switching was developed. This assay detects the appearance of the chimeric switch (S) regions that are characteristic of switched cells. The assay depends upon digestion with restriction enzymes, such as EcoRI, the formation of circular DNAs by ligation and PCR amplification across the ligation joint. With primers that recognize sequences 5' to S μ and 3' to S γ 1, such amplification can only occur in cells that have undergone switching to γ 1 expression. Methods to quantitate this assay have been developed and it has been used to study the precise timing of the DNA deletional events, in normal cells, that occur in switching.

One of the key events that precedes class switching is appearance of a transcript initiated 5' of the S region of the isotype to which the cell will switch. These transcripts are referred to as germ line transcripts and the transcribed exon is designated I. A phosphorothioate anti-sense oligonucleotide complementary to a sequence in I γ 2b proved to a potent stimulant of the expression of the I γ 2b transcript, a powerful stimulant of B cell DNA synthesis and an inhibitor of immunoglobulin secretion. Efforts to understand the mechanism of action of this oligonucleotide are in progress.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Specificity in Immune Responses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	John K. Inman	Section Chief	LI, NIAID
Others:	Andrew Lees	NIH Special Volunteer	USUHS
	Patricia F. Highet	Dept of Medicine	
		Technician	LI, NIAID

COOPERATING UNITS (if any)

Dept Medicine, USUHS, Bethesda, MD (Drs. F. D. Finkelman, J. J. Mond); NIDR, NIH (Dr. F. A. Robey); Virginia Commonwealth Univ (Dr. D. H. Conrad); New York Univ Sch Medicine (Dr. P. Mongini); Food & Drug Admin (Dr. B. Golding).

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.8 (1.0 not paid through NIH)

PROFESSIONAL:

2.0

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard, unreduced type. Do not exceed the space provided.)

A major part of this project is directed toward the design and synthesis of highly specific and efficient immunomodulators. Effector molecules (e.g., monoclonal antibodies directed against designated cell surface proteins) are multiply and covalently linked to soluble polymer carriers of high molecular weight using heterobifunctional reagents. In some studies, protein-protein heteroconjugates have been prepared. New cross-linking reagents have been designed, synthesized and used as part of this project. Special emphasis has been given to improving the chemistry for functionalizing antibody molecules through their polysaccharide moieties and to the cross-linking of these derivatives to functionalized carriers. Collaborative studies employing the immunomodulators have dealt largely with a variety of cell surface factors involved in activation, growth and differentiation of B lymphocytes and presentation of antigen to T cells. For example, it has been shown (Finkelman, Mond et al.) that anti-IgD alone delivers a weak mitogenic signal to resting B cells, but when it is multiply linked to a soluble polymer such as dextran of MW $\geq 200,000$, strong mitogenic stimulation occurs at antibody concentrations ranging from 0.01 to 1.0 mg/ml. These conditions of stimulation are believed to closely approximate normal, T cell-independent antigenic signaling that appears to involve different pathways of transduction than believed to predominate for non-cross-linked signals at higher concentrations. Another objective of this project is to explore the property of general multispecificity of antibodies and other receptors. A study has been completed that measured the distribution of binding constants of a monoclonal antibody for a large collection of diverse compounds (not related to the homologous immunizing hapten). The results strongly support the statistical basis for multispecificity. Quantitative affinity chromatography was used to measure binding constants down to very small values. Techniques for preparing specialized affinity adsorbents were developed for these and other studies. Further work is planned for exploring alternative modes of complementation of ligands in receptor sites and relating these findings to specificity in immune systems and networks.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts (From 12/90)	IRTA Fellow	LI, NIAID
	H.-T. Chen (From 12/90)	Visiting Scientist	LI, NIAID
	P.D. Weinstein (From 6/90)	IRTA Fellow	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID

COOPERATING UNITS (if any)

LOM, NIDR (N. Harindranath); Basel Inst. Immunol. Basel, Switzerland (A. S. Kelus); Dept. Biochem. University of Geneva, Geneva, Switzerland (J.-C. Jaton); Dept. Medicine, Emory University (D. Hayzer); Dept. Microbiol. Loyola Strich Sch. Med. Chicago (K. Knight); Dept. Microbiol. and Immunol. Univ. Illinois Coll. Med., Chicago (W. C. Hanly)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

2.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We use techniques of classical immunogenetics and of molecular biology to study the genetics of rabbit immunoglobulins (Igs) and T cell receptors (Tcr) and to investigate the regulated expression of Ig and Tcr genes during lymphoid cell development.

We reported the thirteenth laboratory observation of a VH-CH recombination (R2M) in the rabbit. All thirteen recombinations have occurred in the male parent. The R2M recombination site maps 3' of the VH genes and 5' of the JH genes as did the sites of three previously characterized recombinations. The R2M site maps 3' of the D1d DH gene which is toward the 3' end of the DH-containing region and is probably in or near stretches of repetitive DNA. There may be two or more "hot-spots of recombination" within or near stretches of repetitive DNA in the DH-containing region of the rabbit.

We obtained the complete genomic sequences of the D β 2 and J β 2 segments associated with a chimeric C β gene present in some rabbits. The rabbit J β 2 cluster has six functional segments, one pseudogene, and a remnant of another pseudogene between J β 2.2 and J β 2.3, equivalent to the one found in man at the same location. The J β 2.5 gene segment of rabbit has lost the splice signal and is a pseudogene unlike its counterparts in man and mouse. The general organization of the gene segments in the D β 2-J β 2 regions of all three species is remarkably conserved over long stretches of DNA sequence; there is higher similarity to human than mouse.

Rabbits were bred at the NIH to produce elevated levels of lambda light chains lacking c21 and expressing only c7. These rabbits were shown to produce mRNA and proteins with sequences corresponding to the products of a previously identified genomic lambda light chain gene, C λ 6. The production of c21 is known to be due to expression of C λ 5. The c21-negative phenotype reflects deletion of a region including J λ 5. The c7-negative phenotype also appears to result from a deletion.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00134-29 LI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

P.I.	Richard Asofsky	Section Chief	LI, NIAID
Others:	J. Little	IRTA Fellow	LI, NIAID
	S. Minato	Visiting Fellow	LI, NIAID
	H.-K. Chu	Visiting Associate	LI, NIAID
	F. Zhao	Visiting Associate	LI, NIAID
	A. E. Brooks	Biological Technician	LI, NIAID
	J. A. Brooks	Biologist	LI, NIAID

COOPERATING UNITS (if any)

W. McCabe & C. Sulis (Infectious Diseases Unit, Boston City Hospital); J. J. Mond (Dept. Medicine, USUHS Med. School); J. Abrams (Dept Immunology, DNAX Research Institute).

LAB/BRANCH

Laboratory of Immunology

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland, 20892

TOTAL MAN-YEARS:

7.0

PROFESSIONAL

5.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Induction of the hybrid B lymphocyte cell line TH 2.2 (BALB/c lymphoma X C57 BL spleen cell) with LPS results in reduction of growth in the line and secretion of IgM and of three cytokines, IL-3, IL-6 and GM-CSF. We have examined growth and cytokine secretion in clonal progeny of individual cells obtained by limiting dilution. Induced cells give rise to large (>5000 cells/well) clones which can be recovered and grown indefinitely, and to microscopically visible (<200 cells/well) clones which are terminal. The clonal frequency in the presence of LPS was less than 5% of the frequency among uninduced cells. Microscopic clones were most often highly specialized, secreting a single product, or at most two. The great majority of the clones making two products released IgM and one or another of the cytokines. This result suggests that the secretory products are produced by terminally differentiated cells with a narrow secretory specificity. The large clones varied greatly in size, over at least a tenfold range. Many clones were recovered which secreted only IgM; a few others secreted only single cytokines. Other clones were recovered which made various combinations of products, including a great many which made all four, and a few which secreted none. Clones of restricted and unrestricted secretory phenotype were recovered and retested. Those of restricted phenotype, including clones which had secreted none of the four products, could be induced by LPS to secrete all, or almost all of the products. Thus the phenotypic restriction is not the result of heritable genetic variation among the cells, but seems, rather, to be a reversible adaptive response to the conditions of culture and induction. The results show that a considerable diversity of response is rapidly generated among these cloned cells. In order to examine whether single cells make single or several products, and to determine if the diversity described above is generated in other cell lines, we have prepared riboprobes containing digoxigenin-labeled UTP (DIG-UTP) for use in studies employing *in situ* hybridization. The probes are detected with enzyme-labeled anti-DIG antibody by standard histochemical procedures.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Receptors, Co-Receptors, and Counter-Receptors for T Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. M. Shevach Section Head LI, NIAID
Others: B. Classen, Staff Fellow, LI, NIAID; K. Roberts, Visiting Associate, LI, NIAID;
D. Wilde, Medical Staff Fellow, LI, NIAID; F. Lynch, Visiting Fellow, LI,
NIAID; C. Brando, Visiting Associate, LI, NIAID; D. Eilat, Visiting Scientist,
LI, NIAID; D. Salomon, Guest Worker, LI, NIAID; L. Ding, Visiting Fellow,
LI, NIAID; M. Rocken, Special Volunteer, LI, NIAID

COOPERATING UNITS (if any)

Biological Resources Branch, NIAID, NIH (J. Coligan)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

10.0

PROFESSIONAL:

8.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies performed over the past 10 years have demonstrated that the interaction of the T cell receptor (TCR) with its specific ligand, peptide-MHC, is only one component of the cascade of events required for the activation of antigen-specific T cells. The primary goals of our studies are to characterize other cell surface antigens (co-receptors) and their soluble or cell-associated ligands (counter-receptors) which play critical roles in cell-cell interaction and the interaction of T cells with their environment (for example, extracellular matrix (ECM)-proteins). We have focused our efforts in two specific areas: 1) We have characterized a prominent subpopulation of $\gamma\delta$ T cells which express the $\text{C}\gamma 4$, $\text{V}\delta 6$ TCR and which appears to recognize an autoantigen expressed on the surface of the activated T cells themselves. However, engagement of this TCR by itself is not sufficient to induce T cell activation as this subpopulation requires the co-receptor function of the vitronectin receptor (VNR) which binds to the RGDS sequence in ECM-proteins. We have produced two soluble forms of the $\text{C}\gamma 4$, $\text{V}\delta 6$ TCR and these novel reagents should allow us to characterize the stimulatory autoantigen and/or the restriction element which regulates activation of this subpopulation of T cells. 2) We have demonstrated that the very early activation (VEA) antigen which has been defined by monoclonal antibody (mAb) H1.2F3 functions as a cell surface receptor for costimulatory signals derived from accessory cells. Non-stimulatory F(ab')_2 fragments of H1.2F3 block all accessory dependent T cell function, but have no effects on accessory cell independent T cell activation. Furthermore, when populations of T cells primed in vivo to two antigens are stimulated in culture by accessory cells and one antigen in the presence of the F(ab')_2 reagent, they cannot be restimulated by the antigen used in the first culture, but mount a normal response to a second antigen. This result suggest that the failure to engage the VEA may result in the induction of clonal anergy. An increase in our understanding of the function of these co-receptors, further characterization of their ligands or counter-receptors, and the availability of unique reagents (mAbs, soluble receptors) should offer us the opportunity to manipulate and regulate ongoing immune responses in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00226-010 LI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts (From 12/90)	IRTA Fellow	LI, NIAID
	H.-T. Chen (From 12/90)	Visiting Scientist	LI, NIAID
	P.D. Weinstein (From 6/90)	IRTA Fellow	LI, NIAID
	P. Fuschioti	Visiting Associate	LI, NIAID

COOPERATING UNITS (if any)

Basel Inst. Immunol. Basel, Switzerland (A. S. Kelus); Dept. Microbiol. Loyola Stritch Sch. Med. Chicago (K. Knight); Dept. Microbiol. and Immunol. Univ. Illinois Coll. Med., Chicago (W. C. Hanly); Dept. Exp. Med. and Bioch. Sci., Univ. of Perugia, Perugia Italy (M. Allegrucci).

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

3.0

PROFESSIONAL

2.0

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We study genes of the rabbit immune system by techniques of molecular biology and immunology. Rabbits of the *Alicia* strain have a mutation (*ali*) that segregates with the immunoglobulin heavy chain (*Igh*) locus and has a cis effect upon the expression of heavy chain variable region genes (VH) encoding the $\alpha 2$ allotype. A relatively small deletion affects a segment containing 3' VH genes, the loss of which leads to the *ali* phenotype. The 3' end of the VH locus probably plays a key role in regulation of VH gene expression in rabbits because the VH1 gene is the target of preferential VDJ rearrangement. This raises the possibility that somatic mechanisms such as gene conversion and hypermutation make major contributions to generating the rabbit's antibody repertoire.

DNAs from homozygous VH-CH recombinant rabbits and from the appropriate non-recombinant parental haplotypes were characterized using Southern blots hybridized with a panel of probes derived from cloned regions of the rabbit immunoglobulin heavy chain gene complex. In all three recombinants, the site was downstream of the entire VH cluster and upstream of the JH cluster within an ~50 kb region containing expanses of repetitive-sequence DNA as well as DH genes.

The rabbit has two isotypic forms of the immunoglobulin kappa light chain, K1 and K2 which probably arose by duplication. In the normal rabbit, only traces of K2 light chains are produced. We used pulsed field and transverse alternating field electrophoresis to obtain megabase maps and found that the two C κ genes are about 1 megabase apart. One explanation for the poor expression of K2, could be great physical distance from V κ genes. However, we found that there are V κ , J κ and C $\kappa 2$ genes within a ~105 kb fragment. Thus physical distance of V κ from C $\kappa 2$ may not be the basis for poor K2 expression. We were interested in looking for second enhancers 3' of the C κ genes because the absence of a 3' enhancer in the K2 locus could explain the preferential utilization of the K1 isotype. A strong region of enhancer activity is found about 7 kb downstream of the C $\kappa 2$ gene. Thus absence of the 3' enhancer is probably not the explanation for low expression of the K2 gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00349-08 LI
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Structure and Function of Murine Class II MHC Genes and Gene Products		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)		
PI:	R. Germain Section Head LI, NIAID	
Others:	L. Racioppi, Visiting Associate, LI, NIAID; C. Layet, Visiting Associate, LI, NIAID; R. Koenig, Visiting Associate, LI, NIAID; S. Sadegh-Nasseri, Senior Staff Fellow, LI, NIAID; A. Fox, Research Technician, LI, NIAID; L-Y. Yu, Research Technician, LI, NIAID	
COOPERATING UNITS (if any) LCMI, NIAID (R. Schwartz); FDA (L. Matis); Univ. of Chicago (A. Sant); Columbia University (N. Braunstein); Basel Institute for Immunol. (F. Ronchese); Univ of Oslo (O. Bakke)		
LAB/BRANCH Laboratory of Immunology		
SECTION Lymphocyte Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS <div style="text-align: center; font-weight: bold;">4.8</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">3.8</div>	OTHER: <div style="text-align: center; font-weight: bold;">1.0</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Class II MHC (Ia) gene products play critical roles in a variety of T and B lymphocyte responses. A combination of immunological, molecular genetic, and biochemical approaches is being used to study the relationship between Ia structure and function. The defect in expression in most cases of "allelically mismatched" α and β chains lies in the failure of assembled heterodimers to efficiently exit the endoplasmic reticulum. Many of these poorly transported dimers can be "rescued" at least in part by co-expression of adequate amounts of the non-MHC encoded invariant chain, a process that is affected by the intracellular localization signal(s) on the cytoplasmic tail of the invariant chain. This same region also controls the efficiency of class II peptide acquisition. <i>In vitro</i> experiments with purified class II demonstrate that conformation is dictated by whether or not appropriate peptides are bound by the NH₂-terminal domains of the $\alpha\beta$ dimer, and that the most stable structure of class II includes such bound peptides. </p> <p> The use of direct peptide binding techniques, together with transfectants expressing mutant class II molecules, has permitted the definition of a local peptide binding subregion in the murine I-E molecule, and the role of specific allelically polymorphic residues in controlling the quantitative and qualitative binding of peptide to this molecule. </p> <p> Finally, mutational analysis has begun to define the site(s) of interaction of Ia with CD4, a molecule that functions as a co-receptor along with the T cell receptor in recognition of class II molecules during T cell activation. Together with the studies on class II folding, transport, and control of peptide binding by the polymorphic domain, these experiments will provide new insight into the molecular mechanisms involved in antigen recognition by and activation of T lymphocytes. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: David H. Margulies Section Head LI, NIAID
Others: Lisa Boyd, Chemist, LI, NIAID; Charles Hoes, Biologist, LI, NIAID;
David H. Chin, Guest Worker (HHMI-Schol), LI, NIAID; Maripat Corr,
Medical Staff Fellow, LI, NIAID; Rosemarie Hunziker, Sr. Staff Fellow,
LI, NIAID; Steven Kozlowski, Medical Staff Fellow, LI, NIAID;
Randall Ribaud, Guest Worker, LI, NIAID; Michael Mage, Sabbatical
Scientist, LB, NCI

COOPERATING UNITS (if any)

LI, NIAID (G. Otten, R. Germain); MB, NCI (T. Takeshita, J. Berzofsky)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

9.0

PROFESSIONAL

7.0

OTHER

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The focus of this laboratory has been the MHC class I molecule, classically considered a molecular heterodimer consisting of a 46 kd polymorphic heavy chain, exemplified by the murine H-2K, D, and L molecules (analogous to the human HLA-A, -B, and -C molecules), that is non-covalently assembled with the 12 kd monomorphic light chain, β 2-microglobulin. Our objectives in these studies have been to examine a number of specific aspects of the biosynthesis, structure, and function of the class I molecule, including: 1) the measurement of the biochemical parameters that govern the binding of the MHC class I molecule to antigenic peptide; 2) the influence of β 2-microglobulin and other serum factors on functional and biochemical binding of MHC class I molecules to antigenic peptides; 3) the expression, folding, assembly, structure, and stability of class I molecules produced in: a) an in vitro RNA-dependent translation system and b) an insect virus/moth ovary cell expression system; 4) the expression folding, assembly, structure, and stability of domain and sub-domain fragments of class I molecules generated by recombinant DNA methods, and expressed in tissue culture cells; 5) the function of soluble MHC class I molecules in a transgenic mouse model system; and 6) the production of single chain MHC class I molecules to examine the role of the heavy chain/ β 2-microglobulin interaction in the function and stability of these molecules and as the basis for high level production in prokaryotic systems.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	L. Racioppi	Visiting Associate	LI, NIAID
	G. Otten	Senior Staff Fellow	LI, NIAID
	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

FDA (L. Matis); BRMP; Basel Institute for Immunology (F. Ronchese)

LABORATORY

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.30

PROFESSIONAL:

1.05

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
 ☐ (b) Human tissues
 ☒ (c) Neither
- ☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antigen-specific T lymphocyte activation occurs through the clonally distributed T cell receptor. The available set of $\alpha\beta$ T cell receptors is dictated by both positive and negative intrathymic selection events. The molecular basis of this selection, in particular, how bias for self-MHC presented peptides is achieved without corresponding deletions of these clones during establishment of self-tolerance, is unknown. Furthermore, the quantitative and qualitative relationships between receptor occupancy and signaling for differentiation, whether in the thymus or of mature T cells in the periphery, is also poorly understood. This project uses cellular and molecular tools to study the development of the T cell repertoire and the activation of T cells upon ligand engagement of $\alpha\beta$ and non-clonal surface receptors.

Co-stimulatory events regulate IL-2 production by CD8 and CD4 T cells. We have demonstrated that the anergic state previously described for Th1 CD4+ T cells also exists from CD8 cytotoxic cells, and can be dissociated from receptor signalling for lytic function. Investigation of a prototypic Th1-type T cell clone specific for cytochrome c and E α E β k unexpectedly revealed that addition of the known peptide ligand recognized by this cell interfered with alloantigen stimulation of IL-2 production by the clone. This inhibition does not appear to be due to simple competition for creation of the alloantigen itself or to over-stimulation of the clone. Rather, it seems that peptide complexes with the mutant class II molecule leads to low affinity (or low efficacy) occupancy of receptors, which interferes with intracellular signaling mediated by the alloantigen-TCR pair. Preliminary data suggest an interference with induction of co-stimulatory activity in the antigen presenting cell. These observations, if they can be generalized and better understood, suggest a previously unappreciated role of low affinity T cell receptor recognition events in regulation of T cell differentiation and activation. This may provide an important clue to the control of thymic selection, as well as the initiation of immune responses. These findings also point to a new approach for the control of autoimmune diseases characterized by oligoclonal T cell responses.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00425-07 LI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T.M. Chused	Senior Investigator	LI, NIAID
Others:	Y.A. Ishida	Visiting Fellow	LI, NIAID
	J.A. Meligeni	Research Associate	
	E.M. Brown	Biologist	LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Transmembrane Signalling Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3.5

PROFESSIONAL

2.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive "feed-forward" and "feed-back" regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. V. Sitkovsky

Head

LI, NIAID

Others: R. Taffs

Staff Fellow

LI, NIAID

A. Filippini

Visiting Fellow

LI, NIAID

T. Sugiyama

Visiting Fellow

LI, NIAID

F. Redegeld

Visiting Fellow

LI, NIAID

M. Piper/Hunter

Research Technician

LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

5.25

PROFESSIONAL

4.25

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The goal of our studies is to understand the biochemical pathways and to identify key proteins (enzymes) that are involved in the activation and the effector phase of T cell-mediated cytotoxicity and exocytosis. It is expected that such knowledge will enable us to selectively manipulate different functional responses of T-cells with specifically designed immunomodulators.

We recently suggested a novel mechanism of cell-to-cell interactions (an "extracellular ATP hypothesis") that may lead to cytotoxicity. According to this hypothesis high local concentrations of extracellular ATP acting alone or in concert with other molecules may induce changes in target cell plasma membrane and, eventually, result in target cell death.

While testing this model we found the following: 1. CTL do indeed accumulate extracellular ATP in a Ca^{2+} -independent manner in response to the crosslinking of TCR complex by mAb; 2. extracellular ATP can kill tumor cells, while CTL themselves are resistant to lytic effects of ATP; 3. high-levels of ecto-ATPase activity are expressed on the CTL, but not on the tumor target cell surface implicating ecto-ATP in the protection of CTL from the lytic effects of extracellular ATP; 4. extracellular ATP kills cells even in the absence of extracellular Ca^{++} ; and 5. both CTL and extracellular ATP are able to induce DNA degradation in TC; and 6. extracellular ATP is most likely acting as a "messenger" in concert with other molecules.

These data indirectly support the model. However, direct evidence for such role of extracellular ATP is still required and work is in progress to develop appropriate reagents. Immunomodulation. The enhancement of the effector responses of CTL by inhibitors of protein phosphatase and by anti-sense RNA for the catalytic subunit of PK-A, provide the first experimental demonstration to support the possibility of being able not only to inhibit but also to enhance the CTL effector response.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

B Cell Stimulatory Factor-1 (BSF-1)/Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: William E. Paul Chief LI, NIAID
Others: R. Seder, Visiting Fellow, LI, NIAID; A. Keegan, Guest Researcher, LI, NIAID;
T. Tanaka, Visiting Fellow, LI, NIAID; J. L. Boulay, Visiting Fellow, LI, NIAID;
D. Agus, Guest Researcher, LI, NIAID; S. Z. Ben-Sasson, Visiting Scientist, LI,
NIAID; M. Plaut, Guest Researcher, LI, NIAID; Z. Grossman, Visiting Scientist,
LI, NIAID

COOPERATING UNITS (if any)

NCI (J. Pierce); Brigham and Women's Hospital (S. Galli and A. Dvorak)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

5.2

PROFESSIONAL

3.7

OTHER

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard, unreduced type. Do not exceed the space provided.)

Interleukin-4 (IL-4) is a potent lymphokine that mediates a wide variety of functions on both hematopoietic and non-hematopoietic cells. Both in vivo and in vitro, it controls the production of IgE antibodies by regulating the process through which B cells switch to the expression of immunoglobulin of that isotype. Among T cells from naive donors, very few (~1/1000) are capable of producing IL-4 in response to a polyclonal stimulant such as immobilized anti-CD3 antibody. Immunization of mice in such a way as to induce high levels of IgE, such as infection with parasitic nematodes or injection of anti-IgD antibody, results in the appearance of large numbers of T cells that can produce IL-4. In vitro priming systems have been developed through which T cells can develop into IL-4 producing cells. These systems depend upon the action of IL-4 itself on precursors of IL-4-producing cells. Such priming can be achieved both by polyclonal stimulants and, using mice transgenic for a T cell receptor specific for cytochrome c, by antigen-specific stimulation.

IL-4 is also produced by FcεRI+ cells in response to cross-linkage of FcεRI, of FcγRIII or to treatment with ionomycin. In bone marrow cell cultures grown for 5-7 days with IL-3, the principal IL-4 producing cell is FcεRI+, c-kit-. Upon isolation, such cells are strikingly enriched in basophils, strongly suggesting that this cell is the predominant hematopoietic cell, other than the T cell, that produces IL-4 in responses to a receptor-mediated stimulation. The capacity of such cells to produce IL-4 is markedly augmented by treatment with IL-3. Current evidence suggests that IL-3 functions by increasing the strength of the signal generated by cross-linkage of FcεRI, as shown by increase in tyrosine phosphorylation of certain substrates in response to FcεRI cross-linkage in mast cells that had been precultured in IL-3.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00520-04 LI

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analysis of T Lymphocyte Responses to HIV Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	W-H. Boehncke	Special Volunteer	LI, NIAID
	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

MB, NCI (J. Berzofsky, T. Takeshita); Nippon Medical School (H. Takahashi)

LAB/BRANCH
Laboratory of Immunology

SECTION
Lymphocyte Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:
.35

PROFESSIONAL:
.35

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To develop therapeutic and vaccine strategies to combat HIV-1, the cause of AIDS, it is essential to understand the cell-mediated immune response to this agent. To pursue this goal, murine model systems for immunization, isolation of antigen-specific T cells, and *in vitro* analysis of T cells specific for HIV-1 proteins have been devised. Restimulation *in vitro* of spleen cells from H-2d mice immunized with recombinant vaccinia virus containing the HIV-1 (IIIB) gp160 gene elicited CD8+ cytolytic T cells specific for amino acids 315-329. This same procedure also was successful in generating CTL specific for the HIV-1 (MN) gp160 and HIV (RF) gp160. All responses were found in H-2d mice, and the CTL elicited were H-2Dd restricted. The determinant involved in the MN and RF responses corresponded to the same region as that involved in the IIIB response, yet the CTL produced were highly specific and did not cross-react on target cells incubated with the different peptides. To understand the relationship among these and other HIV isolates in terms of CTL specificity, variant peptides representing either natural isolates or synthetic combinations of residues from the natural sequences were prepared and used as immunogens or target antigens. These studies showed that the CTL responses to HIV fall into groups related by the chemical nature of key epitopic residues in the inducing peptide. Further, alternation in the peptide structure between immunization and boost broadened the CTL response and suggests a strategy for immunization in man. Related structure-function studies on a class II-restricted determinant have revealed how a small subset of residues controls binding to MHC molecules and how additional residues regulate the epitopic structure necessary for T cell recognition.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. Germain	Section Head	LI, NIAID
Others:	G. Otten	Senior Staff Fellow	LI, NIAID
	J. Sherman	Medical Staff Fellow	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	A. Rinker	Research Technician	LI, NIAID
	L-Y. Yu	Research Technician	LI, NIAID

COOPERATING UNITS (if any)

Molecular Biology Section, LI, NIAID (D. Margulies, R. Ribaud, S. Kozlowski); Mt. Sinai School of Medicine (E. Bikoff)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.55

PROFESSIONAL:

1.55

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

T lymphocytes bearing $\alpha\beta$ receptors respond to complexes of peptide antigen and class I or class II MHC molecules. The way in which protein antigens are transformed into peptides suitable for such binding, the events involved in facilitating such peptide-MHC association, and the intracellular pathways followed by MHC molecules both before and after peptide association are critical to our understanding of T cell immunity.

We previously proposed two distinct pathways for peptide acquisition by class I and class II MHC molecules. To examine the biochemical and cell biological basis for this distinction, and the allele-independent molecular events involved in peptide-MHC molecule association, we have examined normal and mutant cell lines varying in their capacity to generate effective peptide-MHC complexes for the rate of class I and class II molecule assembly and transport, in the presence and absence of known MHC ligand peptides, and under varying growth conditions. In cells defective in transport of peptides into the ER, class I heavy chain- $\beta 2m$ complexes are unstable and inefficiently reach the cell surface. These loosely associated dimers can be accumulated at the cell surface by high concentrations of free $\beta 2m$, revealing an important role for a dynamic equilibrium state that retards class I denaturation/internalization. Free heavy chains on the membrane exist in a transiently re-foldable state, and the combination of peptide and $\beta 2m$ can create native class I molecules from these free heavy chains.

Class II molecules are efficiently assembled and transported in these same cells, indicating no absolute requirement for ER peptides or invariant chain in promoting initial class II dimer assembly and ER egress. However, during intracellular transport, class II dimers alter their stability and conformation in concert with peptide acquisition in (a) post-Golgi compartment(s). This peptide acquisition is inefficient, and many "empty" class II molecules reach the cell membrane. Additional antigen increases the conversion of unstable to stable dimers and enhances overall class II expression. These latter observations provide new approaches for identifying the specific intracellular site(s) of class II peptide binding and for investigating the regulation of intracellular class II transport.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Family of κ B Regulators for Genes in the Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Lenardo	Senior Staff Fellow	LI, NIAID
Others:	A. Kuang	Visiting Student	LI, NIAID
	H. Dang	Student Researcher	LI, NIAID
	S. Kang	Howard Hughes Med. Student	LI, NIAID
	M. Grilli	Visiting Fellow	LI, NIAID
	M. Mead	Guest Researcher	LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Regulation of genes for several lymphokines as well as other molecules involved in the immune response depend on a 10 bp DNA sequence termed, κ B. This sequence binds a growing family of nuclear proteins, several of which are related to the rel oncogene, that are capable of tightly governing transcription of these genes. Importantly, the κ B sequence is found in the human immunodeficiency virus (HIV). A cardinal feature of the κ B sequence is that it permits transcription in a highly regulated fashion- temporally and in appropriate cell-types for specific genes. We are attempting to elucidate how this specific regulation occurs. We have found the IL-2 receptor alpha chain gene enhancer acts specifically in T cells through cooperation of NF- κ B and another gene regulatory protein called SRF.

We have also found that the microheterogeneity in DNA sequence among κ B sites has regulatory significance. By studying normal non-transformed T lymphocyte clones we discovered a novel nuclear complex, termed NF-CYT1 that interacts preferentially with a κ B site in the interleukin-2 gene. The presence of NF-CYT1 in a number of different biological conditions is inversely correlated with IL-2 gene expression in T cells. This suggests it may be a negative regulator. Very significantly, this factor binds to the enhancer region of HIV. We postulate it may have a role in suppressing HIV viral transcription in resting T cells. We also have determined that activation of NF- κ B can occur during antigenic stimulation of T cells independently of T cell receptor signalling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00566-02 LI

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Gene Regulatory Events in T Cell Tolerance and Thymic T Cell Maturation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Lenardo	Senior Staff Fellow	LI, NIAID
Others:	S. Kang	Howard Hughes Med. Student	LI, NIAID
	K. H. Lee	Visiting Fellow	LI, NIAID
	A. Chen-Tran	Research Technician	LI, NIAID
	H. Dang	Undergraduate Student	LI, NIAID
	A. Mermelstein	Summer Student	LI, NIAID
	J. Zúñiga-Pflücker	Jane Coffin Childs Fellow	LI, NIAID

COOPERATING UNITS (if any)

LCMI, NIAID (R. Schwartz, B. Beverly, K. Brorson); BRMP, NCI (A. Kruisbeek)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

5.0

PROFESSIONAL

3.5

OTHER

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Increasing evidence suggests important events in T cell maturation in the thymus and tolerance induction are governed by specific gene regulation. T cell tolerance has been found to occur in immature T cells in the thymus and also extrathymically in mature T cells. Our studies of mature T cell non-responsiveness have shown that expression of the IL-2 gene is disabled by down-regulation of a cellular proto-oncogene that forms part of the AP-1 gene activation complex. Extra-thymic deletion of mature T cells also involves IL-2 which has been found to program these cells for death following antigen receptor occupancy.

In the thymus, precursor cells undergo a complex set of developmental events and emerge as mature T lymphocytes capable of specific antigen recognition. We have found that gene expression varies at different stages of thymic development in a variety of transformed cell lines that represent discrete stages of thymic development. We are focusing on regulation of the genes for IL-2 and its receptor alpha and beta chains. We are also trying to identify gene products associated with clonal deletion in the thymus. Finally we are trying to understand the role of genes, especially cellular proto-oncogenes, in the progression of thymocytes through their maturation pathway.

LABORATORY OF IMMUNOPATHOLOGY

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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF IMMUNOPATHOLOGY, NIAID
October 1, 1990 to September 30, 1991

Herbert C. Morse III
Chief, Laboratory of Immunopathology

The programs of the Laboratory of Immunopathology are directed, in large part, at understanding the mechanisms by which viruses interact with the host immune system to induce various diseases including neoplasms and immunodeficiency. Most of this work is based on studies of murine leukemia viruses (MuLV) and papovaviruses. Analyses of different pathways of hematopoietic differentiation, with particular emphasis on the B cell lineage and oncogenes that influence B cell growth and differentiation represent other major aspects of the program.

Retrovirus-induced immunodeficiency. In the last year, studies of MuLV have focused almost exclusively on analyses of a unique set of ecotropic, mink cell focus-inducing (MCF) and defective retroviruses, termed LP-BM5 MuLV, and the disease they induce, a syndrome characterized by progressive lymphoproliferation and severe immunodeficiency designated mouse AIDS (MAIDS). A series of important observations from the Viral Oncology and Virology and Cellular Immunology sections have significantly furthered our understanding of this disorder.

First, the proximal cause of MAIDS was shown to be a replication-defective MuLV genome that has been molecularly cloned and sequenced. The nonpathogenic ecotropic virus component of the virus mixture has also been cloned and the gag and LTR sequences determined (Chattopadhyay, Fredrickson, Morse, Hartley). The pol and env genes of this virus are greatly truncated and apparently are not translated while the gag gene is of near normal size and encodes a slightly smaller protein than that of nonpathogenic MuLV. The amino acid sequence of the defective virus gag differs most from other gag sequences in its contiguous portions of p15 and p12. Studies of infected mice indicate that the defective virus is expressed in B cells (Chattopadhyay et al) and macrophages (Cheung, Chattopadhyay, Hartley, Morse, Pitha) and possibly in skin (Rosenberg, Morse).

The development of molecular clones of the defective and ecotropic viruses has permitted greater definition of the contributions of the MCF component of the LP-BM5 virus mixture to disease. Studies of mice infected with the defective virus rescued by the ecotropic helper [DEF27(eco)] showed that the course of disease was delayed in comparison to that in mice infected with the uncloned mixture of defective, ecotropic and MCF viruses. However, mice coinfecting with DEF27(eco) and uncloned MCF virus developed disease

with a course similar to that of mice infected with the uncloned viruses (Hartley, Morse, Chattopadhyay).

Second, for B cells studies in vitro, the gag-encoded protein appears to behave like a "superantigen" in stimulating vigorous proliferation of T cells utilizing restricted variable regions sequences of the T cell receptor beta chain (Hügin, Vacchio, Morse). Previously, only certain Staphylococcal enterotoxins and products of some Mycoplasma had been known to have similar properties.

In addition to stimulating massive T cell proliferation, superantigens cause the release of lymphokines that may mediate the pathology associated with infection. Analyses of mice inoculated with LP-BM5 MuLV showed immediate increases in expression of a variety of cytokines including IL-2, -4, -5, -10 and IFN- γ . This pattern of expression is one that might be expected from a mixed Th1/Th2 helper T cell response to the virus. At later times, however, expression was markedly biased to cytokines produced by Th2 cells - IL-4, -5, and -10. The demonstration of enhanced expression of cytokines that stimulate B cell growth and differentiation provides a basis for understanding the progressive lymphoproliferation characteristic of MAIDS. In addition, IL-10 is known to inhibit both Th1 and CD8⁺ T cell function and may explain many features of impaired immune function that occur late in disease (Gazzinelli, Makino, Chattopadhyay, Sher, Hügin, Morse).

Third, results from several studies suggest that resistance to MAIDS exhibited by some strains of mice is related to the activity of CD8⁺ T cells that eliminate cells expressing the defective virus genome. This conclusion was first suggested by analyses of the sensitivity to MAIDS of B6 mice bearing mutations of class I MHC genes that serve as restricting elements for CD8⁺ cytotoxic cells. While some mutations had no effect on the time course of MAIDS, others were associated with significant acceleration or retardation of disease (Makino, Morse, Hartley). In other studies, Southern blot analyses showed that copies of the defective virus genome were not detectable in DNA prepared from spleens of the disease-resistant strain, A/J, at 7 or 12 weeks post infection but were readily detected in DNA from spleens of sensitive B6 mice as early as two weeks post infection (Chattopadhyay, Fredrickson, Morse, Hartley). However, copies of the defective genome were detected in DNA prepared from spleen of A/J mice depleted in vivo of CD8⁺ T cells by treatment with monoclonal antibody to CD8 (Makino, Morse, Hartley). As the gag-encoded protein of the defective virus must be the target of the cytotoxic cells and the p12 portion of this molecule differs most from other MuLV, efforts were made to protect mice from disease by immunization with p12 protein synthesized in bacteria. Although the protein elicited both antibody and proliferative responses from immunized mice, these responses did not protect B6 or B10.BR mice from disease after infection with LP-BM5 MuLV (Tang, Hügin, Hartley, Morse, Chattopadhyay).

Future studies will be directed at understanding the nature of determinants in the defective virus gag protein that elicit immune responses effecting resistance or susceptibility to MAIDS in different strains and further defining the host genetic loci that govern the

course of disease. In addition, mice infected with the virus mixture will be used to study the effects of retrovirus-induced immunodeficiency on infections with other agents that cause severe problems in AIDS such as Toxoplasma gondii and cytomegalovirus.

DNA tumor viruses. Studies in the Viral Pathogenesis section continue to focus on the characteristics of tumor induction by cells transformed in vitro with human adenoviruses, SV40 or polyoma virus. Repeated analyses of animals inoculated with varying numbers of transformed cells have permitted the development of quantitative measures of different tumor cell traits that appear to be independently regulated by the viral oncogenes responsible for their transformation. These oncogene-controlled processes were found to include induction of immortality, control of the threshold dose of cells required for tumor formation, tumor latency and "conditional tumorigenic phenotype". The latter term was coined to indicate the relationship of tumor growth to the state of the host immune system in studies taking advantage of animals with differing immune capabilities [eg. adult vs. newborn vs. congenitally T cell deficient (nude)]. It was found that a novel single graphic display revealing these varying traits could be produced by incorporating data on tumor latency into presentations of the relationship of tumor cell dose and tumor incidence. Comparisons of these "tumor producing dose 50% endpoint curves" for different cell lines indicated that tumor latency was a viral gene-controlled trait which was distinct from that determining the tumor cell dose/tumor incidence relationship (Lewis, Alling, Banks, Soddu, Cook).

Other studies from this section have shown that introduction of the adenovirus 5 E1 gene to highly tumorigenic, spontaneously transformed cells renders them susceptible to lysis by natural killer cells in vitro and eliminates their ability to induce tumors in immunocompetent animals. An understanding of how these cells become susceptible to elimination by the immune system could provide new avenues for development of tumor therapy (Walker, Wilson, Lewis, Cook).

Finally, studies of the relationship between MHC class I gene expression and tumorigenicity of adenovirus transformed cells have yielded results challenging the dogma that reduced expression of the MHC loci is associated with increased tumorigenicity. Employing the quantitative analyses of tumorigenicity described above, transformed cells transfected with class I genes to effect high level expression were found to be more tumorigenic than the parental cells, clearly indicating that lowered class I gene expression is not central to the tumor inducing capacities of adenovirus transformed cells. (Soddu, Lewis).

Oncogenes in B cell neoplasia. The cbl oncogene was captured by an acutely transforming retrovirus that induces pre-B cell and some myeloid tumors in mice and transforms fibroblasts in vitro. Studies of this gene have been directed at defining its genomic organization, the structure of its normal transcripts and evaluating the mechanisms responsible for acquisition of its transforming potential in the viral form. The mouse genome contains two cbl-related sequences. One, termed Cbl-1, is a processed pseudogene

inserted in a LINE sequence (Shapiro, Morse), while the second, Cbl-2 is a complex gene. The human genome contains a single sequence related to Cbl-2. Analyses of murine Cbl-2 showed that the gene is composed of multiple small exons separated by relatively large introns and spans more than 24 kb (Shapiro).

Complete cDNA sequences of the human and mouse genes were determined, demonstrating striking sequence conservation at the amino acid level between species and at both the amino acid and nucleotide level between the murine viral and cellular sequences. Further comparisons of the cDNA sequences with the sequence of v-cbl revealed that the viral gene was generated by a large 5' truncation including a proline-rich region and a leucine zipper-like motif (Shapiro, Morse, Langdon). Other genes with proline rich regions and leucine zippers have been shown to govern transcriptional activity, suggesting that the normal cbl functions by activation genes that restrict cell growth. Unexpectedly, when introduced into a retroviral vector, the complete human cbl cDNA induced transformation of fibroblasts, indicating that the transforming activity of v-cbl could not be ascribed to truncation and arguing again the proposal mentioned above for the function of the gene (Langdon, Hartley, Campbell).

Hematopoietic differentiation. Cell surface markers that distinguish among hematopoietic lineages have proven to be powerful tools for understanding relationships among lineages and the nature of genes important in signaling cell activation (e.g. cytokine receptors) and cellular interactions. LIP-6 is a monoclonal antibody that reacts with most bone marrow cells, pre-B cells, B cells and splenic macrophages but not mature T cells. The possibility that this antibody may recognize a cytokine receptor is suggested by two observations. First, the antibody stimulates proliferation of splenic B cells in the absence of T cells or additional stimuli. Second, incubation of cells with antibodies to the IL-5 receptor or ThB, a member of the Ly-6 gene family, results in enhanced expression of the antigen recognized by LIP-6. Studies are in progress to determine the biochemical characteristics of the protein (Holmes, Morse).

Administrative changes. Dr. Masahiko Makino, a Fogarty Fellow, left the Laboratory after an extremely successful three years to work in the National Institute of Health in Tokyo. Dr. Yao Tang, from Beijing University, joined the Laboratory as a Guest Worker on a fellowship from Boehringer Ingelheim Fonds.

Honors and awards. Dr. Herbert C. Morse III received the PHS Outstanding Service Award.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 0013-28 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Andrew M. Lewis, Jr. Head, VP Section LIP, NIAID

Others: Silvia Soddu Visiting Fellow LIP, NIAID
Yvonne Eyster IRTA Fellow LIP, NIAID
David Alling Collaborator ODIR
Steve Bank Collaborator ODIR

COOPERATING UNITS (if any)

National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado
(J. L. Cook).

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of the elusive goals of studying DNA tumor viruses has been to determine how viral oncogenes convey tumor producing capacities to normal cells. The key to tumorigenicity is generally believed to lie in the process of viral oncogene-induced transformation. However, numerous studies have found transformed cells that are non-tumorigenic and viruses that can transform cells but are unable to induce tumors. Thus, in spite of many years of elegant work on the mechanisms of transformation, the role of viral genes in actual tumor development remains a mystery. A new approach seems to be needed. Based on the concepts 1) that transformation as defined by those changes in normal cells that can be recognized in vitro are only part of the process of tumorigenicity and 2) that the differences in tumor inducing capacity as expressed by DNA virus transformed cells represent oncogene functions that are independent of transformation as recognized in vitro, we have developed such an approach. The basis of this approach lies in the biometric analyses of the relationship between tumor cell dose, tumor incidence and tumor latency. These parameters are the fundamental components of the process by which transformed cells induce tumors when injected into animals. From these analyses, we have developed unique graphical formats for displaying and analyzing the tumorigenic phenotype expressed by transformed cells. Using this format we have found that the tumor inducing capacity of transformed cells is composed of at least 4 distinct viral oncogene controlled processes. Only one of these processes can be detected by studies of cell transformation in tissue culture.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00135-16 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of Immunoglobulin Secreting Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Herbert C. Morse III	Chief	LIP, NIAID
Others:	U.R. Rapp	Senior Investigator	LVC, NCI
	J.H. Pierce	Senior Investigator	LCMB, NCI
	K.L. Holmes	Senior Investigator	BRB, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---------------------------------------------|--------------------------------------------|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE THIS YEAR 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00138-15 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Viruses and Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Herbert C. Morse III	Chief	LIP, NIAID
	J.W. Hartley	Senior Investigator	LIP, NIAID
	K. L. Holmes	Investigator	LIP, NIAID
	T.N. Fredrickson	Research Microbiologist	LIP, NIAID
	R.M.L. Buller	Senior Staff Fellow	LVD, NIAID
	A. Singer	Chief	LEI, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---------------------------------------------|--------------------------------------------|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE THIS YEAR 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO-AI 00205-09 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Herbert C. Morse III Chief LIP, NIAID

COOPERATING UNITS (if any)

Juntendo University, Tokyo, Japan (T. Shirai, K. Okumura)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE THIS YEAR 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00284-10 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Pathogenic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Janet W. Hartley	Head, Viral Oncology Section	LIP, NIAID
Others:	H.C. Morse III	Chief	LIP, NIAID
	T.N. Fredrickson	Research Microbiologist	LIP, NIAID
	S.K. Chattopadhyay	Expert	LIP, NIAID
	M. Makino	Visiting Fellow	LIP, NIAID
	Y. Tang	Guest Worker	LIP, NIAID

COOPERATING UNITS (if any)

FDA (A. Rosenberg); LMM (A. Khan)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

1.4

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project involves the isolation and characterization of C-type murine leukemia viruses (MuLVs), with particular emphasis on biological and molecular factors important for infection of mice, specification of target cells, and induction of neoplastic or other disease. The primary subject of current study is the complex of viruses (LP-BM5 MuLVs) responsible for induction of murine AIDS. Sensitive strains of mice infected with LP-BM5 virus, a mixed stock containing a 4.9Kb replication defective MuLV genome (BM5def) and B-tropic replication competent ecotropic and MCF helper MuLVs, develop progressive lymphoproliferative disease and impairment of immune system functions, the pathogenetic process requiring the defective viral genome and both B cells and CD4⁺ T cells. When virus stocks obtained by co-transfection of cells with molecularly cloned BM5def and helper BM5 ecotropic MuLV DNAs were used to infect mice of the sensitive C57BL strains, disease developed that was in all immunologic and histopathologic respects identical to that induced by the original uncloned virus mixture. Differences noted were delayed onset and progression of disease and lower frequency of spleen cells producing helper virus. Coinfection of mice with additional helper virus, particularly MCF MuLV, resulted in enhancement of the degree of splenomegaly and lymphadenopathy and modest but consistent increase in frequency of ecotropic virus producing cells. Further studies of the molecularly cloned BM5def genome have revealed that while cell lines derived following successful transfection may contain the same size extra-genomic band that is detected using a BM5def probe in Southern blots of lymphoid tissues from infected mice, lines also occur with widely different restriction enzyme patterns of hybridizing fragments. Virus recovered from some of these lines after infection with helper virus induces MAIDS which may be associated with structurally altered genome.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00286-10 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Janet W. Hartley	Head, Viral Oncology Sec.	LIP, NIAID
Others:	M. Makino	Visiting Fellow	LIP, NIAID
	H.C. Morse III	Chief	LIP, NIAID
	T.N. Fredrickson	Research Microbiologist	LIP, NIAID
	Y. Tang	Guest Worker	LIP, NIAID

COOPERATING UNITS (if any)

R. Schwartz, NIAID, D. Murphy, NY State Dept. of Health; I. Egorov, Jackson Labs; M. Zijlstra, Whitehead Inst; R. Melvold, Northwestern Univ Med Sch.

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

2.6

PROFESSIONAL:

1.4

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of genetically based sensitivity and resistance to murine leukemia virus (MuLV) infection and disease induction have centered on BM5 MuLV and MAIDS. For the disease-inducing defective BM5 genome (BM5def) pseudotyped by B-tropic helper virus, the most sensitive mice are those of Fv-1^b genotype and H-2 haplotypes b, f, k, p, q, r, or s. In the insensitive H-2^d and H-2^a strains, resistance is linked to the D end of H-2, suggesting a major role for a protective immune response that is CTL-mediated and thus affected by MHC Class I genes and CD8⁺ T cells. Depletion of the CD8⁺ T cell population in mice of highly resistant strain A/J, following infection with BM5 MuLV, resulted in development of moderately severe disease, increased replication of ecotropic helper virus, and integration of the BM5def genome in cellular DNA. Neither disease nor defective virus integration was observed in mice depleted of CD4⁺ T cells, IL-2 or IFN- γ , although some mice replicated ecotropic virus at a higher level than control infected mice. Further, mutations in both D and K region Class I genes of C57BL (sensitive) mice can alter the course of disease, and tests of C57BL homozygous for a disrupted β 2- microglobulin transgene and thereby deficient for MHC Class I suggest that such mice have enhanced susceptibility to MAIDS. Much evidence indicates that MAIDS is an immunopathologic condition, in which the interaction of MHC Class II genes, CD4⁺ T cells, and BM5def viral antigen are required and in which Class II region effects can influence Class I H-2D-regulated resistance. The results of testing a large number of H-2 recombinant and mutant mouse strains for sensitivity to MAIDS show that I-E α E β ⁺ mice are the most sensitive while I-E α E β ⁻ strains are uniformly sensitive.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00465-06 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Retrovirus-induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Herbert C. Morse III	Chief	LIP, NIAID
Others:	Janet W. Hartley	Head, VO Sec.	LIP, NIAID
	S. K. Chattopadhyay	Expert	LIP, NIAID
	A. W. Hügin	Visiting Associate	LIP, NIAID
	M. Makino	Visiting Fellow	LIP, NIAID
	A. S. Rosenberg	Senior Investigator	CBER DV, FDA

COOPERATING UNITS (if any)

Uniformed Services, University of Health Sciences, Bethesda, MD (C.M. Snapper); Hospital Cantonal Universitaire, Geneva, Switzerland (A. Cerny, S. Izui), Johns Hopkins Cancer Center (P. Pitha)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

3.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is directed at understanding the cellular mechanisms responsible for the development of immunodeficiency in mice infected with a unique set of murine retroviruses, termed LP-BM5 murine leukemia viruses (MuLV). Animals infected with these viruses rapidly become markedly impaired in their ability to respond to a variety of mitogenic or specific antigenic stimuli in vitro. These abnormalities are also evident in vivo as infected mice sequentially lost the ability to reject skin grafts across MHC class II and MHC class I barriers. These immune defects have been found to result from activation of CD4⁺ T cells responding to an antigen expressed by B cells. Analyses of the activation process in vitro showed that the B cell determinant responsible for CD4 stimulation is a component of the gag gene encoded by the defective virus genome present in the virus mixture. This molecule behaves like a superantigen in stimulating T cells with restricted T cell receptor beta chain expression in a CD4- and MHC class II-restricted fashion. Stimulation of this T cell subset appears to induce disease secondary to the release of cytokines as progression of the disease is greatly inhibited by treatment of mice with a drug, cyclosporin A (CSA), that inhibits cytokine production. Analyses of cytokine expression by spleens of infected mice revealed a burst of spontaneous cytokine release (IL-2, IL-4, IL-5, IL-10, IFN- γ) at one week post infection without the need for mitogenic or antigen stimulation. At later times, spontaneous release was not observed, but following stimulation with Con A, a cytokine profile characteristic of Th2- type helper cells was detected. Preferential activation of this CD4 subset may explain the chronic B cell activation and lymphadenopathy characteristic of this disease, mouse AIDS, (MAIDS).

Others:	R.T. Gazzinelli	Staff Fellow	LPD, NIAID
	A. Sher	Senior Investigator	LPD, NIAID
	M.S. Vacchio	Staff Fellow	LEI, NCI
	Y. Tang	Guest Researcher	LIP, NIAID

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00484-05 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kevin L. Holmes	Head, Flow Cytometry Section	BRB, NIAID
Others:	Herbert C. Morse, III	Chief	LIP, NIAID
	L. Staudt	Investigator	DCBD, NCI
	P. Sherle	Staff Fellow	DCBD, NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.25

PROFESSIONAL:

2.0

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- | | | |
|---------------------------------------------|--------------------------------------------|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Further progress has been achieved on the characterization of the antigen identified by the MAb, LIP-6, which is expressed by most bone marrow cells, pre-B and B cells and splenic macrophages but absent on mature T cells. Studies of a LIP-6⁺ Ly-5⁺ B220⁻ bone marrow population show that it is 10-fold enriched for B cell CFU and 20-fold enriched with the addition of IL7, over whole bone marrow. Preliminary experiments suggest that this antigen may be closely associated with the B lineage antigen ThB and the IL5 receptor. LIP-6 can stimulate the in vitro proliferation of normal splenic B cells, maximally after 48 hrs. We have recently generated an anti-LIP-6 MAb of the IgG class, which will be useful for further biochemical and molecular characterization. More recent studies have focused on an antigen, Ly-26, which has been only partially characterized. Initial studies show that it is expressed by B and macrophages lineages, and CD4⁺ cells but absent on CD8⁺ cells. It may also subdivide CD4⁺ cells into two populations. In addition, anti-Ly-26 blocks the binding of anti-Mac-1 (CD11b) antibodies to macrophages, suggesting a relationship between these antigens.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00544-03 LIP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis of Pathogenesis of Murine Leukemia Virus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sisir K. Chattopadhyay	Expert	LIP, NIAID
Others:	Herbert C. Morse III	Chief	LIP, NIAID
	Janet W. Hartley	Head, Viral Oncology Sec.	LIP, NIAID
	Torgny N. Fredrickson	Research Microbiologist	LIP, NIAID
	Masahiko Makino	Visiting Fellow	LIP, NIAID
	S.K. Ruscetti	Senior Investigator	NCI

COOPERATING UNITS (If any)

Johns Hopkins University (P.M. Pitha and S.C. Cheung)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.3

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

LP-BM5 murine leukemia virus, a derivative of Duplan-Laterjet virus, contains a mixture of replication-competent B-tropic ecotropic and mink cell focus-inducing (MCF) viruses and a defective genome that is characterized by combined biological and molecular techniques as the proximal cause of a syndrome, murine AIDS (MAIDS), manifested by lymphoproliferation and immunodeficiency. The ecotropic and 4.9kb defective genome were molecularly cloned and shown, in combination, to induce changes characteristic of MAIDS. Sequence analyses of the 4.9kb defective genome and the LTR and gag gene of the replication competent B-tropic ecotropic virus demonstrated that the defective virus was most likely derived by an initial recombination between the ecotropic virus and endogenous MCF-like sequences followed by deletion and mutation. The most highly conserved region of the 4.9kb virus in relation to the ecotropic parent is in gag with the aminoterminal portion of p12 being the most divergent. A 100bp probe from the defective p12 region was generated. In sensitive C57BL/6 mice the defective virus genome was detected in tissues within 2 weeks of infection but was absent from tissues of the MAIDS resistant strain, A/J, 12 weeks after infection. B cell lineage tumors from mice with MAIDS contained an expressed BM5 defective genome, and clonal integrations of this genome were variably associated with clonal expansions of B cells in infected mice. Protein p12 gag generated in *E. coli* induced high levels of antibodies in both susceptible and resistant mice but failed to induce a protective immune response.

Others: Yao Tang
Ambros Hügin

Guest Researcher
Visiting Associate

LIP, NIAID
LIP, NIAID

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00578-02 LIP

PERIOD COVERED

October 1, 1990 to September 31, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Oncogenes in B Cell Neoplasia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Marjorie A. Shapiro	Staff Fellow	LIP, NIAID
Others:	Herbert C. Morse III	Chief	LIP, NIAID
	J.F. Mushinski	Investigator	LG, NCI
	C.A. Kozak	Microbiologist	LMM, NIAID
	Egerton A. Campbell	IRTA Fellow	LIP, NIAID

COOPERATING UNITS (if any)

Institute of Medical and Veterinary Science, Adelaide, Australia (W. Y. Langdon)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

2.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

The development of neoplasms in vertebrates is often associated with alterations in the structure or expression of cellular proto-oncogenes. We have been characterizing the cbl proto-oncogene which was first discovered in a defective retrovirus that induces pre-B lymphomas *in vivo*. The sequences of 3 cDNA clones (1 human and 2 mouse) show remarkable homology between the two species. One of the murine cDNAs has a 51bp internal deletion which is not due to alternative splicing. There are no nucleotide differences between v-cbl and c-cbl, rather, v-cbl is a truncated form of the gene. The sequences show c-cbl but not v-cbl to contain a leucine zipper. Sequencing of the germline locus has identified 7 exons so far, ranging in size from 120 to 201bp. A clone containing the 5' end of the gene has also been isolated and efforts to determine the regulatory sequences required for cbl expression are underway. cbl is not an immediate early gene and its expression is not regulated upon the addition of mitogens to various cell types. cbl, however, may play a role in the differentiation of certain cell types since terminal differentiation of erythrocytes and embryonal carcinoma stem cell shuts off cbl expression. Constructs containing either v-cbl or c-cbl have been made and will be transfected into F9 EC cells to determine the role of cbl in terminal differentiation. Both constructs have also been shown to transform NIH3T3 cells so it is not yet clear what the functional differences are between v-cbl and c-cbl. Constructs containing either v-cbl or c-cbl in conjunction with the IgH enhancer are being used to develop transgenic mice.

LABORATORY OF IMMUNOREGULATION
1991 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1990 through September 30, 1991

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation

Immunopathogenic Mechanisms of HIV Infection

Over the past several years, the LIR has been involved in studying the mechanisms of immunopathogenesis of human immunodeficiency virus (HIV) infection with particular attention to the modulation of HIV expression by normal endogenous immunoregulatory cytokines. We have previously established that phorbol esters (PMA) or tumor necrosis factor alpha and beta (TNF- α and β) upregulate HIV expression via activation of the cellular transcription factor NF- κ B whereas other cytokines such as interleukin-6 (IL-6) and granulocyte-macrophage colony stimulating factor (GM-CSF) enhance HIV expression at the post-transcriptional level. We have also demonstrated that other cytokines such as interferon (IFN)- α and transforming growth factor (TGF)- β exert suppressive effects on viral production by chronically infected promonocytic cells (U1) at both transcriptional (TGF- β), post-transcriptional (TGF- β), and post-translational (IFN- α) levels. Using the model of TGF- β suppression of PMA induction of HIV expression in U1 cells, we have demonstrated both TNF-dependent and TNF-independent pathways of HIV induction as well as NF- κ B dependent and independent pathways of virus induction and suppression. We have further demonstrated that TGF- β can also exert inductive effects on HIV replication in either acutely infected U937 cells or in primary monocyte-derived macrophages (MDM). IFN-gamma (INF- γ) was shown to have dichotomous effects on HIV expression in that it suppressed PMA-induction of HIV while synergizing with cytokines such as TNF- α in the induction of virus expression.

We have also demonstrated that glucocorticoids (GC) synergized with TNF- α and IL-6 in the induction of HIV expression in U1 cells, and that this synergy resulted from different post-transcriptional mechanisms. It stabilized HIV mRNA with TNF-induction and operated by as yet unidentified post-transcriptional mechanisms in synergy with IL-6.

We studied the modulation of HIV expression by a variety of pharmacologic agents and demonstrated that the anti-oxidants glutathione (GSH), glutathione ester (GSE), and N-acetylcysteine (NAC) blocked the induction of HIV expression by PMA or cytokines. Unlike GSH and GSE, NAC actually blocked transcription of HIV mRNA. Most of this latter effect requires the presence of an intact core enhancer region in the HIV LTR and is likely accounted for by inhibition of NF- κ B. Retinoic acid (RA) suppressed the induction of HIV in a manner strikingly similar to that of TGF- β , acting at both transcriptional and post-transcriptional levels depending on the inductive signal. Based on these data, NAC is already being evaluated in clinical trials in HIV infected individuals and RA is under consideration for clinical evaluation.

The physiologic relevance of cytokine regulation of HIV expression was demonstrated in studies on the role of activated B cells from HIV-infected individuals in the induction of HIV expression in infected T cells and monocytes. B cells from individuals infected with HIV in

contrast to normal controls have been shown to spontaneously secrete elevated levels of TNF- α and IL-6. Furthermore, lymph node B cells isolated from an HIV infected individual also spontaneously secreted elevated levels of both cytokines. These cytokines are not only potentially important in the induction of virus expression by chronically infected cells, but in addition are autoutilized by the B cells themselves in the maintenance of immunoglobulin (Ig) production. B cells from HIV-infected individuals spontaneously secrete elevated levels of virus specific Ig as well as Ig of unknown specificity. The addition of either anti-TNF- α or anti-IL-6 antibodies markedly impairs the secretion of Ig by these spontaneously activated B cells *in vitro*. We have also shown that stimulation of B cells from HIV infected individuals but not normal B cells with the HIV envelope protein gp120 results in both enhanced cytokine and Ig production. The effects of gp120 on B cells from an HIV-infected individual were not mediated via CD4 or B cell Fc receptor. Finally, we have shown that lymph node germinal center have large amounts of HIV which may be important in both the infection of other cells as well as the activation of HIV specific B cells.

Using a modified polymerase chain reaction (PCR) methodology to determine the presence of HIV-specific mRNAs in peripheral blood (PB) cells of infected patients, we have demonstrated that active viral replication occurs at all stages of HIV disease including the early asymptomatic stage. In addition, we have demonstrated a strong association between the presence of mRNA for the gag structural protein of HIV and advanced immunosuppression. The high level of expression of HIV-specific mRNAs, and in particular gag message, in many healthy seropositive individuals may further argue for early initiation of antiviral therapy.

In related studies designed to determine whether HIV-infected circulating CD4⁺ T cells represent an accurate reflection of the total body pool of HIV-infected CD4⁺ cells, we comparatively analyzed the viral burden in PB and lymphoid tissue (lymph nodes, adenoids and tonsils) from the same patients. We demonstrated by quantitative PCR that lymphoid organs are major reservoirs of HIV infection, and lymph node CD4⁺ cells have at least a 10-fold greater frequency of HIV infection than PB CD4⁺ cells.

We have extended our previous studies on the infection of thymic precursor cells by HIV and have examined the effect of HIV on the thymic microenvironment. Thymic epithelial (TE) cells were cultured and it was determined that these cells were neither susceptible to HIV infection nor altered in their expression of thymocyte-trophic cytokines and adhesion molecules. We demonstrated that normal human serum induced secretion of IL-6 by TE cells, that the induction of IL-6 was partially due to IFN- γ in serum, and that supernatants from TE cells maintained in human serum upregulated HIV replication in chronically HIV-infected cells. These findings suggest that TE cells may actually participate in the promotion of intrathymic spread of HIV.

In studies related to those of TE cells, epidermal keratinocytes have been shown to produce multiple cytokines and growth factors in response to various stimuli, the strongest of which is ultraviolet (UV) irradiation. We have shown that UV irradiation of normal human keratinocytes induces the production of TNF- α in quantities sufficient to induce HIV expression in co-cultures with chronically infected promonocytes and T cells. In view of the fact that Langerhans cells have been reported to be infected with HIV *in vivo*, these findings may have implications for understanding dermal pathology in HIV infected individuals.

Previous studies in our laboratory have demonstrated the ability of the CD34⁺ subset of bone

marrow progenitor cells to be infected with HIV *in vitro*. We have studied freshly isolated CD34⁺ bone marrow cells from 74 Zairian and American HIV seropositive patients and have found that 30% of these patients have HIV infection of the progenitor cell subset as assessed by either viral isolation via co-culture or PCR analysis of the purified cell populations. Since this is the first report of *in vivo* infection of bone marrow precursor cells, it is potentially important to our understanding of the hematologic abnormalities found in HIV infected individuals.

To further study the interactions of HIV and hematopoietic precursor cells, we have utilized the E88 cell line, a chronically HIV infected spontaneously transformed human fetal thymocyte line. Subclones derived from these cells are of two major types: constitutive producers of high levels of HIV virions or non-producers, and attempts to down or up modulate viral expression with various agents have been unsuccessful to date. All of the clones are able to transactivate a transfected HIV-LTR without the addition of stimulating factors, resulting in high rates of transcription. These clones will provide useful tools for the further study of cellular factors which act on the LTR to upregulate virus expression in chronically infected immature cells.

We have studied potential mechanisms of HIV-mediated cytopathicity and viral spread by examining the role of leukocyte adhesion molecules in these processes. We have demonstrated that leukocyte integrin LFA-1 is required for cell fusion and syncytia formation in human CD4⁺ T lymphocytes infected with HIV. In fact, cell fusion and syncytia formation did not occur in CD4⁺ T lymphocytes genetically deficient in LFA-1 expression infected either with HIV-1 or HIV-2. In contrast, the lack of expression of LFA-1 did not influence cell-to-cell transmission of HIV. In addition, we have demonstrated that ICAM-1 and ICAM-2, the two natural ligands described for LFA-1, are not the counterreceptors for LFA-1-dependent syncytia formation suggesting the existence of a third ligand for LFA-1 or a direct interaction of LFA-1 with HIV envelope.

In studies aimed at delineating the mechanisms of progressive depletion of CD8⁺, HIV-specific cytolytic T lymphocytes (CTLs), we demonstrated that CD8⁺ T cells can be infected with HIV *in vitro* in the presence of HIV-infected CD4⁺ T cells or HLA-matched target cells. These results, if confirmed *in vivo*, would suggest that infection of CD8⁺ CTLs occurs during the process of HIV-specific cytolytic activity and may be a potential mechanism of the progressive diminution of HIV-specific CD8⁺ CTLs consistently noted during the course of HIV infection.

In order to dissect further the molecular mechanisms by which HIV kills CD4⁺ T cells, we have engineered CD4⁺ T cell lines expressing individual or groups of HIV-1 genes, and analyzed signalling abnormalities in HIV-infected cells. T cell lines transfected to express the envelope glycoproteins grew normally but were altered in their ability to fuse. The co-culture of envelope-expressing cells with naive CD4⁺ cells led to syncytium formation, cell death, and dramatic alterations in intracellular signalling pathways as assessed by the tyrosine phosphorylation of intracellular substrates. HIV-infected cultures demonstrated similar changes in tyrosine phosphorylation, particularly the induced phosphorylation of a 30 kilodalton (kd) substrate correlating with syncytium formation and cell death, as well as increased phosphorylation of a 95 kd substrate. In studies designed to initiate adoptive immunotherapy for HIV, we have cloned, sequenced and expressed the antibody genes isolated from a human anti-HIV gp41 monoclonal cell line. These antibody genes were genetically modified to donate their variable region combining sites onto T cell receptor (TCR) constant regions, generating a chimeric antibody/TCR capable of expression in T lymphocytes. (Poli, Schnittman, Pantaleo, Bressler, Stanley, DeMaria, Butini, Graziosi, Kehrl, Rieckmann, Biswas, Cohen, Tani, Lane, Fox, Fauci, LIR/NIAID; Orenstein,

Dept. Path., GU; Kotler, St. Luke's Hosp., NY, NY; de Fougerolles, Springer, Harvard; Haynes, Singer, Wichard, Duke; Kessler, NMRI; Kapita, Musengala, Zaire).

Regulation of HIV Gene Expression

Using site directed mutagenesis and computational analysis we have comprehensively analyzed the distribution of sequence-specific information throughout the REV responsive element (RRE), the target sequence for the REV protein of HIV-1. In contrast to previous investigators we found that sequence-specific information is widespread throughout the RRE, both within and without the primary REV binding domain. The finding of sequence-specific information outside the primary REV binding domain suggests that this region may be specifically bound by cellular factors involved in the REV response. Inside the REV binding domain, which consists of three stems extending from a central base-paired region, IIb, we found that sequence-specific information is about equally distributed amongst the stems. The most critical sequence-specific information in the entire molecule, however, lies in the IIb region at the center of the REV binding domain. This region may act as a nucleation center for REV protein polymerization along the RNA molecule or may be involved in the specific binding of cellular factors. Using this information, and information we previously generated on the structure of the RRE, we have designed a series of RNA probes to study the binding of REV to the RRE and to identify cellular factors involved in the REV response. Initial studies have detected a nuclear activity which promotes REV binding to the RRE. Studies are now underway to study the specificity of this interaction and to characterize, identify and clone the nuclear factor(s) involved.

Also using the information we have generated on the sequence- and structure-specificity of the RRE, we have designed a series of mutant provirus derivatives of HXB2 which have impaired RRE's but which have no alterations of the envelope amino acids encoded by the overlapping env gene. Two such proviruses with mutations in the IIb region of the RRE have been tested and found to differ from the wild type HXB2 with respect to their cell-type specific tropism. In other studies, we have found that vif mutations in different parental HIV strains cause alterations in cellular tropism which are not clearly reflected in the tropisms of the parental strains. By exchanging genetic segments amongst the vif mutants being studied we have determined that at least some of these effects are due to determinants which map to a region of the genome including the env, tat, vpu, rev and nef genes. We are now making point mutations to identify these other determinants. These studies should lead to an improved understanding of tropism in general and the vif mechanism in particular. (Dayton, Dayton, Powell, Pantaleo, Butini, Poli, Fauci, LIR/NIAID).

Neuropathogenic Mechanisms of HIV Infection

We have demonstrated that human astrocytes and two cell lines derived from human astrocytes are capable of secreting cytokines which have been shown to regulate HIV expression: IL-6, TGF- β and GM-CSF. The secretion of all three cytokines is stimulated in time- and dose-dependant manner by IL-1. IL-1 was found to stimulate TGF- β secretion by oligodendrocytes and microglia. These results suggest that the secretion of cytokines by glial cells may stimulate, at least in part, HIV expression in brain.

Pre-existing IL-1 was found dramatically increased whereas TGF- β was induced in tissues from HIV-infected individuals compared to seronegative individuals without neuropathology or

brain disease. The HIV-infected individuals studied displayed a broad range of neurological abnormalities characteristic of AIDS. All tissues from HIV-infected individuals displayed reactive astrogliosis but contained no perivascular infiltrates nor detectable HIV antigens (except in one case). Both TGF- β and IL-1 were expressed by astrocytes and TGF- β was also expressed by microglia. IL-1 was also present in blood vessel endothelium whereas TGF- β was not. These results are consistent with those obtained *in vitro* and suggest that cytokines may be involved in the neuropathogenesis of HIV infection. However, the expression of these cytokines is not unique to HIV infection. The changes in the levels of IL-1 and TGF- β observed in AIDS brain were also observed in brains from patients with fever and several other disorders. These results suggest that these cytokines may be common mediators of the brain's response to virus and other pathogenic insults rather than being specific to HIV infection. Nevertheless since cytokines such as TGF- β alter HIV expression in monocytic cells, they may at least in part determine the level of HIV expression in brain.

We have demonstrated that murine brain cultures can become infected with a mixture of murine leukemia viruses known to cause immunodeficiency syndrome in mice. The data obtained *in vivo* and *in vitro* suggest that both microglia and astrocytes become infected. The data indicate that infected cells harbor a defective viral genome which has been previously demonstrated to be a critical component for disease induction. This animal model provides an opportunity for studying the molecular basis of retrovirus-mediated neuropathogenesis. (Vitkovic, da Cunha, Ehrenreich, Fauci, LIR/NIAID; Brightman, Major, NINDS/NIH; Sei, NIDDK/NIH; Flanders, Sporn, Johns Hopkins Sch. Med.; Jannotta, George Washington Sch. Med.; Mersel, Neskovic, INSERM Unite 44, Strasbourg, France).

The Immune Response to HIV and Related Retroviruses

We have continued our studies on cell mediated immune responses to two of the retroviruses causing disease in man, namely human immunodeficiency virus type 1 (HIV-1) and human T lymphotropic virus type 1 (HTLV-1). We have demonstrated that in HTLV-1 infected patients with the neurological disorder tropical spastic paraparesis (TSP), high levels of virus-specific CTLs are found both in the peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid (CSF). These CTL are CD8⁺ and major histocompatibility complex (MHC)-Class I restricted. CTL to HTLV-1 in patients with TSP are predominantly directed against the regulatory protein tax. Using limiting dilution cloning techniques and mitogenic stimulation, we have generated HIV-1 and HTLV-1 specific CTL clones. We have mapped two new CTL epitopes within HTLV-1 tax that are restricted by HLA-A2 and HLA-B14 with synthetic peptides, the first CD8⁺ CTL epitopes reported to HTLV-1. We have identified the amino acids within two CTL epitopes of HIV-1 and HTLV-1 that are important for binding to MHC-Class I proteins and recognition by the T cell receptor. We have identified HTLV-1 pX region RNA in autopsy tissue from the central nervous tissue of patients with TSP by *in situ* hybridization. We have shown that CTL to HIV-1 and HTLV-1 can inhibit viral production and have found that culture supernatants from antigen activated CTL clones to HIV can inhibit replication of HIV in CD4⁺ cells from seropositive patients. We have found that an HIV-1 specific clone transferred to severe combined immunodeficient mice (SCID) reconstituted with human cells can impede replication of HIV-1 in some animals. We have shown that HIV-specific CTL clones can be activated with a specific peptide antigen to induce calcium influx, and mRNA transcription, protein production, and release of tumor necrosis factor and interferon-gamma. (Koenig, Fauci, Fox, LIR/NIAID; Jacobson, McFarlin, Biddison, NI/NINDS; Rosenberg, Yanelli, Alexander, Surgery/NCI; Woods, Newell, Brewah, MedImmune, Inc.; Mosier,

Torbett, Medical Biology Institute).

Clinical Trials in HIV Infection

A series of clinical trials have been carried out both in normal volunteers and in patients at all stages of HIV-1 infection. In the first human trial of a candidate AIDS vaccine, a dose-escalating study was completed in healthy non-infected homosexual or bisexual human volunteers in which participants were immunized with the gp160 envelope precursor protein of HIV-1. Particularly at the higher doses, this recombinant protein was found capable of inducing group specific T lymphocytes directed toward the HIV-1 envelope and was also capable of raising titers of neutralizing antibodies as high as 1:8.

Separate phase I trials of 3'-azido-2',3'-dideoxyuridine (AZDU) and recombinant CD4-Immunoadhesion (rCD4-IgG) were conducted in which both agents demonstrated no significant toxicities but also failed to show any significant antiviral efficacy as manifested by measurable changes in surrogate markers. It was determined that remarkably low doses of GM-CSF were capable of reversing the neutropenia caused by combination therapy with IFN- α and zidovudine (ZDV). Moreover, addition of this marrow-sparing cytokine did not appear to impair the antiretroviral activity of the combination. A randomized trial comparing the long-term effects of early therapy with ZDV versus IFN- α versus the combination of the two drugs in asymptomatic HIV-infected patients completed its targeted accrual of 180 patients. Inasmuch as the first 60 patients have been enrolled on this study for longer than one year's duration, preparation of the first formal interim analysis of the safety and efficacy data generated thus far was also begun. Phase I/II trials of ZDV plus IFN- α , ZDV plus IL-2, IFN- α plus IL-2, and DDI plus IFN- α were continued. Preliminary analysis of the combination of DDI plus IFN has revealed a significant antiviral effect both as primary therapy and as a salvage treatment for patients failing other antiretroviral combinations, although toxicity of the two-drug combination can be significant. Phase 1 pharmacokinetic and safety studies of L-697,639 and L-697,661, two new promising HIV-1-specific antiretroviral agents, were performed and showed that both agents were orally bioavailable and well-tolerated. A randomized, double-blinded, placebo-controlled 12-week phase II study of L-697,661 versus ZDV in plasma viremic HIV-infected individuals was initiated using surrogate markers as efficacy parameters. A phase 1 study of oral and intravenous therapy with NAC, and a dose-ranging phase 1 pharmacokinetic and safety study of CD4-Pseudomonas Exotoxin (sCD4-PE-40), in HIV-infected patients were begun.

A masked, randomized, delayed therapy controlled trial of foscarnet in AIDS patients with non-sight-threatening cytomegalovirus peripheral retinitis clearly demonstrated this drug to be of benefit in delaying progression of retinitis. An antiretroviral effect of the drug in these patients was also demonstrated. The hydroxynaphthoquinone BW566C80 was shown to be both safe and effective for the treatment of mild to moderate episodes of *Pneumocystis carinii* pneumonia (PCP) as well as for salvage therapy of central nervous system (CNS) toxoplasmosis. A multi-center phase II/III study of the efficacy of BW566C80 versus trimethoprim-sulfamethoxazole for treatment of PCP, and a phase 1 study of BW566C80 in the treatment of cryptosporidial/microsporidial diarrhea, were also begun. Phase 1 analysis of a new oral macrolide, clarithromycin, established the oral bioavailability and maximally-tolerated dose of this agent. Based upon these findings, a study of its efficacy in the treatment of MAI infection was initiated. As supported by strong data from a murine model of Toxoplasma infection, a study of the efficacy of another macrolide, azithromycin, as salvage therapy in CNS Toxoplasmosis was also initiated.

An Immunotherapy protocol involving active immunization of HIV-1 infected individuals with HIV-1 gp160 showed this immunogen to be well-tolerated and capable of inducing significant increases in lymphocyte blastogenesis to recombinant antigen. In a pilot study involving combination chemotherapy with zidovudine, interferon-alpha, and rCD4-IgG coupled with PB lymphocyte and bone marrow transfers to infected twins from uninfected gp160-primed syngeneic donors, preliminary evidence of an antiviral effect has already been seen in at least one of the three twin pairs being studied. (Lane, Fauci, R. Davey, Zunich, Walker, Herpin, Boenning, Haneiwich, Metcalf, V. Davey, LIR/NIAID; Masur, Kovacs, Falloon, Polis, Henderson, CC/NIH; Nussenblatt, NEI)

International AIDS Activities

In two related studies in Kinshasa, Zaire, we demonstrated that AIDS is the leading cause of death with HIV seroprevalence in 43% of adult deaths. We also demonstrated that 40% of 1200 female prostitutes in Kinshasa and 14% of 546 Nigerian prostitutes were HIV-1 seropositive, and that incident infections (3%/year) were strongly correlated with genital ulcers and chlamydial infections. In studies of perinatal infection involving over 600 children born to HIV infected mothers in Zaire and Haiti, we documented a 28% transmission rate of HIV-1 infection and a cumulative mortality rate of 70% in the first 2 years of life. Of surviving children during the first year of life, a beneficial effect of immunizations against commonly occurring childhood infectious diseases such as measles was observed despite the lower immunogenicity in HIV-1 seropositive individuals. In virologic studies we demonstrated that no single diagnostic assay was reliable in diagnosing a perinatally acquired HIV infection within the first month of life, but that by three months, HIV culture, PCR and IgA each had a high positive predictive value which further improved at 6 and 9 months of age. Additional studies demonstrated the presence of neutralizing antibody to the V3 loop region of HIV-1_{mn} in HIV-infected individuals in the U.S., Zaire and Brazil, but we were unable to document an association between the presence of these neutralizing antibodies and perinatal transmission rates. In contrast, perinatal transmission was independently associated with decreased number of CD4 cells, and chorioamnionitis. Additional virologic and immunologic studies further defined the prognostic importance of p24 antigenemia following acid hydrolysis, the role of cytokines, particularly TNF- α and activated memory T-cell subsets in the natural history and immunopathogenesis of HIV infection. (Quinn, Brown, Fauci, LIR/NIAID; Ryder, CDC, Piot, Colebunders, Institute of Tropical Medicine, Antwerp, Belgium; Hook, John Hopkins).

Isolation and Characterization of Genes Important in Human B Cell Function

Using subtractive hybridization techniques we have identified a group of genes which are expressed in B cells and not T cells or expressed at much higher levels in B cells than in T cells. One such cDNA clone has been shown to encode for the B cell membrane protein CD22 which we refer to as BL-CAM or B lymphocyte cell adhesion molecule. Current studies are directed at understanding the role of CD22 as a mediator of B-B interactions and understanding why it is expressed solely in B lymphocytes (see below). We are currently evaluating 3 other subtracted cDNA clones currently designated as clones 10, 11, and 34. Clone 34 is constitutively present in B cells and if present in T cells is present at very low levels. Clones 10 and 11 are both activation genes in B cells but can also be induced at low levels in T cells. cDNAs for each of these genes have been sequenced and final confirmation of the sequences is in progress. Clone

11 is predicted to encode for a membrane protein while no membrane spanning regions have been found to date in the predicted proteins from the other clones.

The CD20 promoter has been isolated and sequenced from a genomic clone which contains the 5' end of the CD20. CD20 promoter CAT constructs have been made and transfected into B and T cell lines. An element in the CD20 promoter has been identified which confers B cell specific expression on a heterologous promoter. In addition, a B cell specific DNA binding protein has been identified which interacts with this sequence. Southwestern blots and UV crosslinking studies have identified an approximately 60 kd protein which binds to this sequence from the CD20 promoter. Current studies are aimed at characterization of the bases which are essential in binding and characterizing the binding protein. Preliminary evidence suggests that the CD20-D binding protein is the B cell specific transcription factor Oct-2. Similarly a genomic clone spanning the promoter region of BL-CAM has been isolated and sequenced. The 5' intron and exon boundaries have been mapped. We are currently mapping the 3' intron and exon boundaries and beginning a functional analysis of the promoter region.

We have isolated and characterized 2 new human homeobox containing genes, HB24 and HB9. Both these genes are expressed in hematopoietic progenitor cells and activated lymphocytes. Genomic clones for HB9 and HB24 have been isolated and partially sequenced. The transcriptional start sites and promoter regions have been identified for both genes. A murine equivalent of the HB9 gene has been isolated from a murine genomic library and partially sequenced. Three cDNA clones have been isolated from a murine B cell cDNA library with the HB9 and HB24 cDNA. While none of these clones correspond to the human HB9 and HB24 genes they appear to be related genes. The HB24 cDNA has been permanently transfected into both a human B and T cell line. The T cell transfectant, Jurkat-HB24, expressed a number of activation genes including IL-2 and IL-2 receptor- α (IL-2R α) which were not present in the parent or an anti-sense transfectant. Co-transfection of HB24 and IL-2R α promoter constructs revealed two HB24 responsive regions in the IL-2R α promoter. Furthermore, the Jurkat-HB24 transfectant was noted to proliferate more rapidly than the controls. The use of anti-sense oligonucleotides revealed that the increase in proliferation was directly attributable to overexpression of HB24. Many of these phenotypic changes noted in the HB24 T cell transfectant were also noted in a B cell HB24 transfectant. The HB24 homeobox gene is increased in CD34 positive cells following exposure to growth factors. The addition of HB24 anti-sense oligonucleotides to CD34 cells stimulated with IL-3 and GM-CSF significantly decreased DNA synthesis as measured by thymidine incorporation. (Deguchi, Kozlow, Rieckmann, Thevenin, Hong, Fauci, Kehrl, LIR/NIAID).

Regulation of Human B cell Function by Soluble Factors

We have continued our studies of the role of IL-6 and TNF- α in B cell function. We have shown that the inhibition of Ig secretion by TGF- β is not secondary to an inhibition in either the production of TNF- α or IL-6 nor to an inhibition in either TNF- α or IL-6 receptor expression. We have shown both normal CD5 positive and CD5 negative B cells isolated from tonsils are capable of synthesizing and secreting both IL-6 and TNF- α . We have continued our studies of the effect of TGF- β on B cell Ig production including studies of TGF- β on transcription factors in B cells. Studies with the 3' kappa light chain enhancer have revealed that TGF- β markedly suppresses its activity in transfected B cells and current studies are directed at identifying the important cis-sequences. (Rieckmann, Fauci, Kehrl, LIR/NIAID).

Production of Endothelins by Hematopoietic Cells and Astrocytes

Endothelins are potent vasoconstrictor and mitogenic peptides originally isolated from endothelial cells which we have demonstrated are synthesized and secreted by mast cells, monocytes and macrophages. Studies with astrocytes have revealed an autoregulatory loop whereby triggering of endothelin receptors can induce endothelin production. In contrast, while monocytes synthesize and secrete endothelins they do not possess endothelin receptors. Mast cells similar to astrocytes possess both receptors for endothelin and secrete endothelin. However, distinct from astrocytes, they express a selective endothelin receptor which binds only endothelin-1 with high affinity. Current studies are examining the potential role of endothelins in the pathogenesis of disease characterized by blood vessel pathology. (Ehrenreich, Rieckmann, Fox, Hoffman, Metcalf, Kehrl, Fauci, LIR/NIAID); Anderson, Coligan, BBR/NIAID).

The Genetic Response of Primary T Lymphocytes to an Activation Signal: Control of Proliferation and Immune Competence

We have cloned, sequenced and characterized a number of selected genes from our collection of cDNAs representing genes rapidly inducible in human T cells with mitogens or antigen. Included in this group are two cytokines, a membrane receptor related to one of importance in signal transduction during killing by natural killer (NK) cells, three zinc finger proteins which constitute their own family of zinc finger genes and which is most related to the Wilms tumor suppressor gene, a steroid receptor-like gene, a protein with homology to phosphatases, a ras oncogene related protein and two rel-related κ B DNA-binding proteins. We continue to investigate structural, functional and regulatory aspects of these genes and their products.

The mitogenically induced zinc finger genes 225, 591 and 133 are all highly related in their zinc finger domains, but differ completely elsewhere. 133 is a recently isolated novel member of this family. 225 in particular, and 591 to a more limited degree are inducible by human T cell leukemia virus (HTLV) transformation of cells. This transactivation is not dependent on T cells as a human osteosarcoma cell line carrying the HTLV genome also upregulated 225. In addition, the HTLV tax product appears to upregulate the 225 gene by itself. To better understand the potential role these genes may play in cellular proliferation and in tumorigenesis if deregulated in their expression, we have cloned two novel *Drosophila* genes with almost identical zinc finger domains. These genes are developmentally regulated which may shed some light on their roles and, by inference, also on this family of human genes.

We have previously cloned the precursor gene for the p50 subunit of the biochemically described classical NF- κ B complex. We have now cloned an additional mitogen-induced gene designated rik which resembles p50 but encodes a distinct product. This gene shares with p50 a rel-related domain and a cell-cycle domain. Furthermore, this gene is capable of binding κ B sites *in vitro*, clearly marking it as a member of the NF- κ B family of proteins. Rik also heterodimerizes with other members of the family, in particular with relB, a novel c-rel-like protein which lacks cell cycle repeats. Finally the rik product in concert with relB can transactivate a κ B-site dependent promoter driving the CAT reporter gene in transiently transfected cells. This demonstrates for the first time that completely novel functional NF- κ B complexes can exist which do not contain any component of the classical biochemically described p50/p65 complex nor do

they contain c-rel. (Siebenlist, Bours, Brown, Wright; LIR/NIAID; Kelly, LP/NCI; Kassis, DBB/FDA; Bravo, Squibb Institute of Medical Research).

Clinical, Immunopathogenic, and Therapeutic Studies in the Vasculitides and Other Immune-Mediated Diseases

We have expanded our studies of the immunopathogenic and therapeutic aspects of the entire spectrum of the vasculitides. We have continued studies that demonstrate the efficacy of daily low dose cyclophosphamide (CP) and glucocorticoids (GC) for these diseases. However, long-term toxicity from CP in 50% of patients, in conjunction with a 50% disease relapse rate has encouraged evaluation of alternative therapies. We have demonstrated the initial efficacy of intermittent high dose intravenous CP, while also indicating that such therapy does not have the ability to sustain long-term remission. Reports by other investigators have suggested that trimethaprim/sulfamethoxazole is a useful therapeutic agent for Wegener's granulomatosis (WG). We have not been able to substantiate those results in our own patients.

We have studied treatment with weekly low dose methotrexate (MTX) and GC in patients with WG and Takayasu's arteritis. Eighteen patients with WG have received MTX, including 10 who had previously received CP but discontinued that agent because of toxicity (6) or relapse (4). Seventy-eight percent of WG patients have achieved a MTX-induced remission and 64% have so far successfully discontinued GC therapy. Fifteen patients with Takayasu's arteritis have also been treated with MTX and GC. These patients had previously either been GC-unresponsive or unable to achieve complete remission with GC therapy. In 7 instances, the addition of a cytotoxic agent (CP in 6 cases, azathioprine in 1 case) also failed to provide GC-free remissions. Eight of 15 Takayasu's arteritis patients thus far treated with MTX have achieved GC-free remissions and 5 additional patients who are in the process of tapering GC are taking 50% less GC than had previously been possible. Apart from one patient having to discontinue MTX because of presumed drug-induced pneumonitis, this agent has been relatively well tolerated. The ultimate role of MTX in the treatment of these diseases is uncertain because of the small number of patients studied thus far and the short period of follow-up (mean period for WG = 10 months, and for Takayasu's arteritis = 20 months). Patients with these diagnoses and other forms of systemic vasculitis will continue to be enrolled in each of these studies. It is obvious that consistently curative therapy of these diseases is not likely to occur without a better understanding of pathophysiologic events.

We have utilized endothelial cell (EC) culture systems to study selected features of the vasculitides. Takayasu's arteritis is an idiopathic inflammatory disease of large arteries which involves predominantly the aortic arch and its branches. Takayasu's arteritis occurs almost exclusively in women during their reproductive years. This demography suggests a role for estrogens in the pathogenesis of vascular inflammation. Because large vessels express estrogen receptors, we investigated the influence of 17- β -estradiol on leukocyte adhesion to cultured human umbilical vein ECs. We have demonstrated that estradiol has important regulatory functions in EC-leukocyte-extracellular matrix interactions which probably contribute to the pathogenesis of Takayasu's arteritis.

We have also utilized EC culture systems in an attempt to identify serum cytotoxic factors in patients with systemic vasculitides. Using an *in vitro* angiogenesis model (D. Grant, Cell 1989;58;933), we found, quite unexpectedly, that certain serum proteins from patients with

systemic vasculitides more strongly stimulated EC differentiation into capillary-like structures than did normal human sera. Although more intense during active vasculitis, serum angiogenesis activity persisted after clinical remission. Characterization of the angiogenesis factor(s), including N-terminal sequencing of these proteins demonstrated that they were haptoglobin and haptoglobin-related protein. Stimulation of angiogenesis is a newly recognized biological function of haptoglobin. We suggest that its persistent elevation in systemic vasculitides may be an important means of compensating for ischemia by formation of collateral vessels. The potential applications of natural angiogenic substances are worthy of exploration in a variety of ischemic conditions, in addition to the vasculitides. (Hoffman, Leavitt, Kerr, Cid, Fauci, LIR/NIAID; Travis, LP/NCI; Fleischer, CC/NIH; Kleinman, LDBA/NIH; Grant, LDBA/NIH; Hallahan, NIAID/NIH; Cotch, Kaslow, DMID/NIH).

Immunopathogenesis of *Chlamydia trachomatis* Infections

Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop rapid diagnostic assays and to examine the pathogenesis of chlamydial infection in experimental animal models. In screening over 2000 men and women attending STD clinics, chlamydia genital infections continue to be common with infection rates of 10%-15%. Chlamydia infections tend to have the characteristics of a prevalent infection since infection rates vary little by either sex, reason for clinic attendance, number of sex partners or the patient's relationship with their sexual partners. Chlamydial cervical infections in women were higher in contacts (26%) than in non-contacts (16%). Chlamydial infection rates were even higher in women who had been contacts of men with gonorrhea with rates as high as 32%, suggesting that co-infection with *N. gonorrhoeae* and *C. trachomatis* are quite frequent in this population. Detection of chlamydia species by PCR and subsequent genotyping by restriction enzyme analysis have been developed. In addition to differentiation of *C. pneumoniae*, *C. trachomatis* and *C. psittaci*, we have demonstrated that nearly three-quarters of *C. trachomatis* serovars are comprised of D, E and F with the remainder comprised of I, J, and K serovars. Serovar distribution did not correlate with clinical presentation, concurrent gonococcal infection or concordance among sexual couples. In a field study of trachoma in Tanzania, PCR detection of chlamydia genome was demonstrated to have a threefold increase in sensitivity compared to DFA, and a semi-quantitative PCR technique was utilized to monitor response to therapy in over 200 children with trachoma. In an experimental animal model where viability of *C. trachomatis* can be monitored, we have demonstrated persistence of chlamydial DNA by PCR in monkeys who have become culture negative. This persistence of chlamydial DNA may possibly contribute to either a sustained hypersensitivity response or a latent state which is inhibited by the presence of neutralizing antibody and cell - mediated immunity. Finally, the 16s rRNA of *C. pneumoniae* was sequenced and compared to *C. psittaci* and *C. trachomatis*, demonstrating that *C. pneumoniae* is more closely related to *C. psittaci* than *C. trachomatis*. Additional studies utilizing primer sets from the 16s rRNA sequence are planned for PCR detection of *C. pneumoniae* as an etiologic agent of pneumonia in pediatric, adult and geriatric populations. (Quinn, LIR/NIAID).

Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR have received a number of awards and honors. Dr. Fauci continues to serve on a number of committees of scientific and administrative importance such as the Institute of Medicine Roundtable for the Development of Drugs and Vaccines against AIDS. He is also chairman of the NIH AIDS Executive Task Force as well as a member of the U.S. Public Health Service AIDS Task Force and the PHS AIDS Executive Committee.

This past year, Dr. Fauci became an advisory editor of The Journal of Experimental Medicine. He continues his role on the Editorial Board of The Journal of Immunopharmacology, Clinics in Immunology and Allergy, EOS, Cellular Immunology, The Journal of Molecular and Cellular Immunology, AIDS Research and Human Retroviruses, and AIDS Patient Care. He serves on the Advisory Board of Immunopharmacology and Immunotoxicology, Clinical Immunology and Immunopathology, and the Journal of Clinical Immunology. He is on the Editorial Advisory Council of La Ricerca in Clinica e in Laboratorio. He also continued his duties as Associate Editor (Allergy and Immunology) of The American Journal of Medicine and as the Consulting Editor for North America for Thymus.

This past year, Dr. Fauci became an associate editor of "AIDS, Etiology, Diagnosis, Treatment, and Prevention." He continues as an editor of "Harrison's Principles of Internal Medicine," "Transactions of the Association of American Physicians," "Current Therapy in Allergy, Immunology and Rheumatology," and "Harrison's Principles of Internal Medicine - Companion Handbook." He is an associate editor of "Current Therapy in Internal Medicine." In addition, he is an editor along with Dr. John I. Gallin of "Advances in Host Defense Mechanisms."

Several members of the LIR were asked to deliver major named or invited lectures during the year. Dr. Fauci delivered several prestigious lectures including the Memorial Lecture of the Silver Jubilee of the Japan-U.S. Cooperative Medical Science Program in Tokyo, the 19th Annual Charles W. Lacaillade Lecture of St. John's University, and the commencement address at The University of Texas Southwestern Medical Center at Dallas. He was the keynote speaker at the Seventh International Lymphokine Workshop and the annual meeting of the Medical Society of Delaware and the invited plenary speaker at the Third (Republic of) San Marino International Conference on Biomedical Research. Dr. Fauci was also an invited symposium speaker at the Second University of Texas Southwestern Symposium on the Physician's Role in Society and the American Association for the Advancement of Science Annual Meeting. In addition, he delivered the annual address to the New York Academy of Medicine. He was an invited plenary lecturer at International Herpesvirus Workshop, the annual Cent Gardes Symposium in Paris, the annual meeting of the International Society of Hematology and the American Society of Hematology, and the First annual RCMI International AIDS Symposium. Finally, Dr. Fauci delivered the plenary lecture at the opening ceremony of the VIIth International Conference on AIDS in Florence, Italy.

Dr. Poli delivered a lecture at the EC/F.E.R.S./MRC Workshop on Immunodeficiency in HIV-1 Infection in Anugraha, Engelfiel Green Surrey, United Kingdom. Dr. Pantaleo delivered an invited lecture at the American Society for Microbiology annual meeting. Dr. Stanley delivered grand rounds at the University of Arkansas for Medical Sciences in Little Rock. Dr. Siebenlist was an invited speaker at the "Hinterzartener Kreis" meeting, at which 25 eminent scientists gathered to discuss important areas of ongoing research and consider directions for the future in biology and

medicine.

Dr. Leavitt gave a seminar on vasculitis at the American Academy of Allergy and Immunology annual meeting, conducted a postgraduate course in rheumatology at the Cleveland Clinic, and was a visiting professor and delivered grand rounds at Abington Memorial Hospital.

Dr. Hoffman delivered grand rounds at the University of Pennsylvania Graduate Hospital and The Johns Hopkins University. He was a visiting professor and also delivered grand rounds at the Columbia University College of Physicians and Surgeons and Mary Imogene Bassett Hospital. He also received the NIAID Director's Award.

Dr. Koenig has been appointed for a two year term as an Associate Editor of the the Journal of Immunology.

The 1990 Science Citation Index ranked Dr. Fauci as the eighth most-cited scientist among 1.3 million scientists publishing in the time period 1981-1988. He received other honors including the Humanitarian Award of the Human Rights Campaign Fund Federal Club of Houston, the First International Chiron Prize for Biomedical Research from the Scuola Superiore di Oncologia e Scienze Biomediche (Genoa, Italy) and the Scuola Internazionale di Oncologia e Medicine Sperimentale (Rome, Italy), the Presidential Award of the New York Academy of Sciences, and the Fifth Annual Gene Frey Memorial Award for Medicine from the Whitman-Walker Clinic in Washington, DC.

Dr. Fauci was elected as a Fellow in the American Academy of Arts and Sciences and received an honorary fellowship from the New York Academy of Medicine. He also received two honorary degrees: Doctor of Medicine and Surgery, Honoris Causa, from the Università di Roma, "La Sapienza" and Doctor of Science, Honoris Causa, from St. John's University, Jamaica, NY.

Administrative, Organization, and Other Changes

The major theme of the LIR continues to be the delineation of the cellular and molecular mechanisms of regulation of the human immune response in normal and disease states. The vast majority of the activities of the LIR currently focus on AIDS, particularly the immunopathogenesis of HIV infection. The size of the laboratory in space, slots and resources has been stable over the past year.

In the Immunopathogenesis Section, Drs. Guido Poli and Giuseppe Pantaleo have been promoted from Visiting Associate to Visiting Scientist. Both are on a tenure track. Drs. DeMaria and Butini have both returned to Italy after 2 year fellowships. Dr. Peter Rieckman has returned to Germany after a 2 1/2 year fellowship. Dr. Steven Schnittman has left the LIR to join the Division of AIDS of NIAID as Chief of the Medical Branch. Dr. Scott Koenig has left to join Med-Immune, a biotechnology firm, as a senior Scientist. Drs. Shirley Lee, Lawrence Fox and Drew Weismann have joined the section following a year on clinical rotation. Dr. Oren Cohen begins his clinical rotation in 1991 and will enter the laboratory at the end of the year. Dr. Gail Kerr has joined the Clinical Vasculitis Unit.

In the Clinical and Molecular Virology Section, Dr. Gail Scully and Dr. Huan Tian have joined the section.

Dr. H. Clifford Lane was appointed Clinical Director, NIAID.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00537-04 LIR

PERIOD COVERED
October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Immunopathogenic Mechanisms of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A.S. Fauci Chief LIR, NIAID

Others: G. Poli Visiting Scientist LIR, NIAID
S. Schnittman Senior Staff Fellow LIR, NIAID
G. Pantaleo Visiting Scientist LIR, NIAID
P. Bressler Senior Staff Fellow LIR, NIAID
S. Stanley Medical Officer LIR, NIAID

COOPERATING UNITS (if any)
LCP, NCI (M. Sporn, A. Roberts); LMM, NIAID (M. Martin); Dept. Pathol., GWU (J. Orenstein); Dept. Virol., Georgetown (B. Fernie); PRI, Frederick, MD (M. Baseler); St. Luke's Hosp., NY, NY (D. Kotler); Center for Blood Research and Harvard Med. Sch., Boston, MA (A. de Fougerolles, T. Springer);

LAB/BRANCH
Laboratory of Immunoregulation

SECTION
Immunopathogenesis Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS 16	PROFESSIONAL 12	OTHER 4
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CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is directed at delineating the cellular and molecular mechanisms of the immunopathogenesis of human immunodeficiency virus (HIV) infection. We have continued to investigate the inductive and suppressive effects of immunoregulatory cytokines on HIV expression. Using a model of transforming growth factor beta (TGF- β) suppression of phorbol myristate acetate (PMA) induction of HIV expression in chronically infected promonocytic cells (U1) we have demonstrated both tumor necrosis factor (TNF)-dependent and TNF-independent pathways of HIV induction as well as NF- κ B dependent and independent pathways of virus induction and suppression. We demonstrated that the anti-oxidants glutathione (GSH) and N-acetyl cysteine (NAC) could block the induction of HIV expression by PMA or cytokines. Unlike GTH, NAC actually blocked transcription of HIV mRNA. Retinoic acid suppressed induction of HIV in a manner strikingly similar to that of TGF- β suggesting its possible use as a therapeutic agent. We demonstrated that glucocorticoids synergized with TNF- α and interleukin-6 (IL-6) to induce HIV expression from U1 cells by different post-transcriptional mechanisms. Using a modified polymerase chain reaction assay to detect HIV-specific mRNAs we demonstrated that active viral replication occurs at all stages of HIV disease including the early asymptomatic stage. In followup of our previous observations that thymocyte precursors were susceptible to HIV infection, we observed that thymic epithelial (TE) cells are not susceptible to HIV infection. However, normal human serum induces TE cells to secrete IL-6 which upregulates the expression of HIV in infected cells suggesting that these cells might actually promote the intrathymic spread of HIV. We demonstrated that CD34⁺ bone marrow progenitor cells from a subset of seropositive individuals most of whom have advanced immunosuppression are infected with HIV in vivo. We demonstrated that lymphoid organs are major reservoirs of HIV and lymph node CD4⁺ cells have at least a 10x greater frequency of infection than peripheral blood CD4⁺ cells. Finally, LFA-1 regulates HIV-mediated fusion and syncytia formation in human CD4⁺ cells.

Others:	A. DeMaria	Guest Researcher	LIR, NIAID
	L. Butini	Guest Researcher	LIR, NIAID
	C. Graziosi	Visiting Associate	LIR, NIAID
	H.C. Lane	Senior Investigator	LIR, NIAID
	J. Kehrl	Senior Investigator	LIR, NIAID
	U. Siebenlist	Expert	LIR, NIAID
	A. Dayton	Senior Staff Fellow	LIR, NIAID
	P. Rieckmann	Guest Researcher	LIR, NIAID
	P. Biswas	Guest Researcher	LIR, NIAID
	C. Fox	Senior Investigator	LIR, NIAID
	C. Brown	Senior Staff Fellow	LIR, NIAID

Cooperating Units:

Duke Univ. Med. Ct., Durham, NC (B. Haynes, K. Singer, L. Whichard); NMRI, Bethesda, MD (S. Kessler); Mama Yemo Hospital, Kinshasa, Zaire (B. Kapita, L. Musengela); Dept. Pathol., Albert Einstein Med. Ctr., NY, NY (W. Hatch, W. Lyman); PB, NCI (P. Pizzo); LVD, NIAID (B. Moss); LTCB, NCI (S. Colombini, P. Lusso); Cornell Univ. Med. Ct., NY, NY (A. Meister).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00585-02 LIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Pathogenic Mechanisms in Human Immunodeficiency Virus and Other Retroviral Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	H.C. Lane	Senior Investigator	LIR, NIAID
Others:	D.I. Cohen	Expert	LIR, NIAID
	Y. Tani	Visiting Associate	LIR, NIAID
	G. Scully	Clinical Associate	LIR, NIAID

COOPERATING UNITS (if any)

CBMB, NICHD (L. Samelson); New York Univ (S. Zolla-Pazner); UCSan Diego (S. Hedrick)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

5.5

PROFESSIONAL

3.5

OTHER

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

In order to dissect the molecular mechanisms whereby human immunodeficiency virus (HIV-1) functionally alters or kills CD4+ T lymphocytes Jurkat cell lines transfected to express different HIV-1 proteins were established. Cells constitutively expressing functional gp120 and gp41 showed no direct alterations in their cell growth but did not spontaneously fuse. In contrast, HIV-infected cells or HIV-envelope transfected cells could be induced to form syncytium and die upon co-culture with naive cells. Such infected or transfected cells undergoing cell fusion and cell death displayed dramatic alterations in their intracellular signalling pathways as evaluated by changes in tyrosine phosphorylation of intracellular substrates. These phosphorylations include the induction of tyrosine phosphate on substrates of 95 and 30 kilodaltons (kd), the latter event displaying kinetic correlation with syncytium formation and cell death. Constructs for the inducible or constitutive T cell expression of HIV-1 nef, vpu, rev, and tat were prepared to permit the functional evaluation of each of these HIV-1 genes both in vitro and in the scid/hu mouse model. Peripheral blood CD4+ T lymphocytes infected in vitro with HIV-1 were found to give rise to CD4-/CD8- gamma/delta (γ/δ) T lymphocytes that did not express interleukin-2 (IL-2) following stimulation. In studies on the peripheral blood mononuclear cells of patients with HIV infection an increased proportion of cells expressing the γ/δ T cell receptor were identified. The heavy and light chain antibody genes derived from two human anti-HIV envelope gp41 monoclonal cell lines were cloned, sequenced, and functionally expressed in recipient B cell lines. The genes from one of the two monoclonal lines conferred anti-gp41 specificity to transfected cell lines. The heavy and light chain variable region genes from this antibody (98-6) were genetically linked to the T cell receptor (TCR) constant α and β regions, respectively to generate chimeric antibody/TCR genes capable of expression in T lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00587-02 LIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regulation of Gene Expression of the Human Immunodeficiency Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A.I. Dayton	Senior Staff Fellow	LIR, NIAID
Others:	E.T. Dayton	Staff Fellow	LIR, NIAID
	D.M. Powell	Graduate Student	LIR, NIAID
	J. Lim	Summer Student	LIR, NIAID
	T. Jeang	Senior Staff Fellow	LMM, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID

COOPERATING UNITS (if any)

LMB, NCI (J.V. Maizel); LMB, NCI (B. Shapiro); Univ. Colorado (D. Konings); LTCB, NCI (R. Gallo); LTCB, NCI (M. Klotman); DMV (B. Baroudy); Inst., Cincinnati, OH (J.N. Gamble); James N. Gamble Inst., Cincinnati, OH (G. Kotwal).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

3

PROFESSIONAL

2

OTHER

1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have comprehensively analyzed the distribution of functionally critical sequence-specific information throughout the RRE, the target sequence for the REV protein of human immunodeficiency virus (HIV-1). We found that sequence-specific information is widespread throughout the RRE, both within and without the primary REV binding domain. The finding of sequence-specific information outside the primary REV binding domain suggests that this region may be specifically bound by cellular factors involved in the REV response. Further we found that sequence-specific information is about equally distributed amongst the stems of the REV binding domain. The most critical sequence-specific information in the entire molecule lies at the center of the REV binding domain, in 11b. Sequence specificity here is clearly found on both strands of the RNA and was identified at the level of single bases. This region may act as a nucleation center for REV protein polymerization along the RNA molecule or may be involved in the specific binding of cellular factors. Using this information, and information we previously generated on the structure of the RRE, we have designed a series of RNA probes to study the binding of REV to the RRE and to identify cellular factors involved in the REV response. Initial studies have detected a nuclear activity which promotes REV binding to the RRE. Studies are now underway to study the specificity of this interaction and to characterize, identify and clone the nuclear factor(s) involved. In other work, we have initiated studies on the possible interaction of the vif protein of HIV-1 with other viral genes. HIV clones with vif mutations in different viral genetic backgrounds have different cellular tropisms which do not clearly mirror parental tropisms. Exchanging sequences between two vif mutants with divergent tropisms determined that some of the sequences responsible for the differences lie in a large envelope-containing region of the genome. We have also accumulated evidence that the RRE can contribute to cell-type specific viral tropism.

Others:

L. Butini	Visiting Scientist	LIR, NIAID
G. Pantaleo	Visiting Scientist	LIR, NIAID
S.M. Schnittman	Senior Staff Fellow	LIR, NIAID
C. Graziosi	Visiting Scientist	LIR, NIAID
A.S. Fauci	Chief	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00562-03 LIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Neuroimmunology and Neuropathogenesis of HIV infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: L. Vitkovic Expert LIR, NIAID

Others: A.S. Fauci Chief LIR, NIAID
A. da Cunha Visiting Associate LIR, NIAID
H. Ehrenreich Guest Researcher LIR, NIAID

COOPERATING UNITS (if any)

NINDS (M. Brightman, E. O. Major), NIDDK (Y. Sei), NCI (K.C. Flanders, M.B. Sporn), The Johns Hopkins School of Medicine, Baltimore, MD (W.R. Tyor), The George Washington University School of Medicine (F.S. Jannotta), INSERM Unite 44, Strasbourg, France (M. Mersel, N. Neskovic).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4

PROFESSIONAL

3

OTHER

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have demonstrated that human astrocytes and two cell lines derived therefrom are capable of secreting cytokines known to be able to regulate human immunodeficiency virus (HIV) expression: interleukin-6 (IL-6), transforming growth factor-beta (TGF-β) and granulocyte macrophage-colony stimulating factor. The secretion of all three cytokines is stimulated in time- and dose-dependant manner by IL-1. IL-1 was found to stimulate TGF-β secretion by oligodendrocytes and microglia. These results suggest that the secretion of cytokines by glial cells may stimulate, at least in part, HIV expression in brain. Pre-existing IL-1 was found to be dramatically increased whereas TGF-β was induced in tissues from HIV-infected compared to seronegative individuals without neuropathology or brain disease. The HIV infected individuals whose brains were assayed displayed a broad range of neurological abnormalities characteristic of AIDS. All tissues from HIV-infected individuals displayed reactive astrocytosis but contained no perivascular infiltrates nor detectable HIV antigens (except in one case). Both cytokines were expressed by astrocytes and TGF-β was also expressed by microglia. IL-1 was also present in blood vessel endothelium whereas TGF-β was not. These results are consistant with those obtained in vitro and suggest that cytokines may be involved in the neuropathogenesis of HIV infection. However, the expression of these cytokines is not unique to HIV infection. The changes in the levels of IL-1 and TGF-β observed in AIDS brain were also observed in brains from patients with fever and several other disorders. These results suggest that these cytokines may be common mediators of the brains response to virus and other pathogenic insults rather than being specific to HIV infection. Nevertheless since cytokines such as TGF-β alter HIV expression in monocytic cells, they may at least in part determine the level of HIV expression in brain. We have demonstrated that murine brain cultures can become infected with a mixture of murine leukemia viruses known to cause immunodeficiency syndrome in mice. The data obtained in vivo and in vitro suggest that both microglia and astrocytes become infected. The data indicate that infected cells harbor a defective viral genome which has been previously demonstrated to be a critical component for disease induction. This animal model provides an opportunity for studying the molecular basis of retrovirus-mediated neuropathogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00586-02 LIR
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) Immune Response to the Human Immunodeficiency Virus and Related Retroviruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S. Koenig	Guest Researcher LIR, NIAID
Others:	A.S. Fauci C. Fox	Chief Expert LIR, NIAID LIR, NIAID
COOPERATING UNITS (if any) NI,NINDS (S. Jacobson, D. McFarlin, W.E. Biddison); Surgery, NCI (S. Rosenberg, J. Yanelli, R. Alexander); MedImmune, Inc. (R. Woods, A. Newell, A. Brewah); Medical Biology Institute (D. Mosier, B. Torbett).		
LAB/BRANCH Laboratory of Immunoregulation		
SECTION Immunopathogenesis Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	3	PROFESSIONAL: 1 OTHER: 2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Our studies have focused on <u>cell mediated immune responses</u> to two of the retroviruses causing disease in man, namely <u>human immunodeficiency virus type 1 (HIV-1)</u> and <u>human T lymphotropic virus type 1 (HTLV-1)</u> . We have demonstrated that chronic infection with HIV-1 may result in high levels of circulating <u>cytotoxic T cells</u> (CTL) in freshly separated peripheral blood mononuclear cells (PBMCs) of healthy seropositive donors. In HTLV-1 infected patients with the neurological disorder <u>tropical spastic paraparesis</u> (TSP), high levels of virus-specific CTL are found both in the PBMC and cerebrospinal fluid (CSF). These CTL are <u>CD8⁺</u> and <u>MHC-Class I restricted</u> . CTL to HTLV-1 in patients with TSP are predominantly directed against the regulatory protein <u>tax</u> . Using limiting dilution cloning techniques and mitogenic stimulation, we have generated HIV-1 and HTLV-1 specific CTL clones. We have mapped two new CTL epitopes within HTLV-1 tax with <u>synthetic peptides</u> , the first CD8 ⁺ CTL epitopes reported to HTLV-1. We have identified the amino acids within two CTL epitopes of HIV-1 and HTLV-1 that are important for binding to major histocompatibility complex-Class I proteins and recognition by the T cell receptor. We have identified HTLV-1 in autopsy tissue from the central nervous system of patients with TSP by <u>in situ hybridization</u> . We have shown that CTL to HIV-1 and HTLV-1 can inhibit viral replication or expression of viral proteins. We have found that an HIV-1 specific clone transferred to <u>severe combined immunodeficient mice</u> (SCID) reconstituted with human cells can impede replication of HIV-1 in some animals. We have shown that HIV-specific CTL clones can be activated with a specific peptide antigen to induce calcium influx, and mRNA transcription, protein production, and release of <u>tumor necrosis factor</u> and <u>interferon-gamma</u> .		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00390-08 LIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Investigation of the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H.C. Lane	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	R.T. Davey Jr.	Medical Officer	LIR, NIAID
	R. Walker	Medical Officer	LIR, NIAID
	E. Seidel	Medical Officer	LIR, NIAID
	M. Easter	Nurse Practitioner	LIR, NIAID

COOPERATING UNITS (if any)

CC, NIH (H. Masur, J. Kovacs, J. Falloon, M. Polis, D. Henderson); NEI, NIH (R. Nussenblatt)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

7.5

PROFESSIONAL:

7.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An intensive effort was directed toward studying the preventive and therapeutic aspects of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). Immunization of healthy volunteers to the gp160 envelope protein of HIV-1 was capable of inducing group specific T lymphocytes directed towards the envelope and titers of neutralizing antibodies as high as 1:8. Phase I trials of AZDU and rCD4-IgG failed to demonstrate efficacy in HIV infection. Low-dose GM-CSF reversed the neutropenia caused by interferon-alpha (IFN- α) plus zidovudine (ZDV) without impairing the antiretroviral activity of the combination. A randomized trial comparing therapy with ZDV versus IFN- α versus the combination in 180 patients with early HIV-1 infection completed accrual and will be undergoing interim analysis. A controlled trial of foscarnet demonstrated significant benefit in patients with AIDS-related cytomegalovirus retinitis. Phase I/II trials were continued of ZDV + IFN- α , ZDV + IL-2, IFN- α + IL-2, and DDI + IFN- α . BW566C80 was shown to be effective for mild to moderate episodes of pneumocystis carinii pneumonia (PCP) as well as for salvage therapy of central nervous system (CNS) Toxoplasmosis. A multi-center phase II/III study of BW566C80 versus trimethoprim-sulfamethoxazole for treatment of PCP, and a phase 1 study of BW566C80 for treatment of cryptosporidial/microsporidial diarrhea, were also begun. Phase 1 studies of L-697,639 and L-697,661 established the safety and oral bioavailability of both agents. A randomized, double-blinded, phase II study of L-697,661 versus ZDV was initiated using surrogate markers as efficacy parameters. Phase 1 studies of N-acetyl cysteine and CD4-Pseudomonas Exotoxin (sCD4-PE-40) in HIV-infected patients were initiated. A phase 1 analysis of clarithromycin established the oral bioavailability of this agent and a study of its efficacy in MAI-infected patients was initiated. A study of azithromycin as salvage therapy in CNS Toxoplasmosis was also begun. Immunotherapy protocols involving active immunization of HIV-1 infected individuals with HIV-1 gp160 or p24 were continued, and passive immunotherapy utilizing peripheral blood lymphocytes and bone marrow from gp160 primed donors was also instituted.

Others:

C. Boenning
S. Haneiwich
B. Herpin
V. Davey
J. Metcalf

Project Coordinator
Project Coordinator
Project Coordinator
Project Coordinator
Biologist

LIR, NIAID
LIR, NIAID
LIR, NIAID
LIR, NIAID
LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00361-09 LIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

International Studies on the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
Other:	A.S. Fauci	Chief	LIR, NIAID
	C. Brown	Expert	LIR, NIAID

COOPERATING UNITS (if any)

CDC (W. Heyward); Institute of Tropical Medicine, Antwerp, Belgium (P. Piot); Johns Hopkins University (E. Hook, N. Halsey).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

2.5

PROFESSIONAL

1.5

OTHER

1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The acquired immunodeficiency syndrome (AIDS) has become a global pandemic with over 366,000 cases reported from 162 countries. A major focus within our unit has been defining the unique epidemiologic, clinical, virologic and immunologic features of human immunodeficiency virus type 1 (HIV-1) infection in Africa and other tropical countries. In two related studies in Kinshasa, Zaire, we demonstrated that AIDS is the leading cause of death with HIV seroprevalence in 43% of adult deaths. We also demonstrated that 40% of 1200 female prostitutes in Kinshasa and 14% of 546 Nigerian prostitutes were HIV-1 seropositive, and that incident infections (3%/yr) were strongly correlated with genital ulcers and chlamydial infections. In studies of perinatal infection in over 600 children born to HIV infected mothers in Zaire and Haiti, we documented a 28% transmission rate of HIV-1 infection and a cumulative mortality rate of 70% in the first two years of life. Of surviving children during the first year of life a beneficial effect of immunizations against commonly occurring childhood infectious diseases was observed despite the lower immunogenicity in HIV-1 seropositive individuals. In virologic studies we demonstrated that no single diagnostic assay was reliable in diagnosing perinatally acquired HIV infection within the first month of life, but that by 3 months, HIV culture, polymerase chain reaction and IgA each had a high positive predictive value which further improved at 6 and 9 months of age. Additional studies demonstrated the presence of neutralizing antibody to the V3 loop region of HIV-1_{mn} in HIV-infected individuals in the U.S., Zaire and Brazil, but we were unable to document an association between the presence of these neutralizing antibodies and decreased perinatal transmission rates. In contrast, perinatal transmission was independently associated with decreased number of CD4 cells and chorioamnionitis. Additional virologic and immunologic studies further defined the prognostic importance of p24 antigenemia following acid hydrolysis, the role of cytokines, particularly TNF- α , and activated T-cell subsets in the natural history and immunopathogenesis of HIV infection. Further studies will continue to examine virologic and immunologic aspects of human retroviral infections internationally.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00210-11 LIR

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Immunoregulation of Human Lymphocyte Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	J.H. Kehrl	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	Y. Deguchi	Visiting Associate	LIR, NIAID
	H. Ehrenreich	Guest Researcher	LIR, NIAID
	C. Fox	Senior Scientist	LIR, NIAID
	J.X. Hong	Visiting Associate	LIR, NIAID

COOPERATING UNITS (if any)

BRB, NIAID (R. Anderson, J. Coligan); LCP, NCI (A. Roberts, S.J. Kim, M. Sporn); LCI, NIAID (A. Kirschenbaum, D. Metcalf); Mount Sinai Medical Center, NY (Vesna Najfeld, Lloyd Mayer); Georgetown Medical Center, Wash. D.C. (P. Katz).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

6

PROFESSIONAL:

4

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

We have continued our studies of the mechanisms of B lymphocyte activation, proliferation, and differentiation at both the molecular and cellular levels. Studies of the role of soluble factors in the regulation of B and T cell activation have been continued with a particular emphasis on several cytokines which are made by B cells themselves. Studies of the role of transforming growth factor-B (TGF- β) in the regulation of B cell immune function have progressed and we have shown that exposure to TGF- β markedly impairs the transcription of kappa and lambda light chain. Our studies with B minus T subtracted cDNA clones have progressed. Initial studies investigating the expression and probable function of one of the subtracted clones, BL-CAM, have been completed. Current studies are directed at mapping the transcriptional start site(s) and preparing constructs to study the promoter. Three other subtracted clones are in various stages of analysis. We are also isolating B cell genes which are expressed either exclusively or predominantly in the germinal center region. We have largely completed the analysis of the CD20 promoter and have characterized a site in the promoter which confers B cell specific expression on a minimal promoter. We have continued studies with 3 human homeobox containing genes; HOX 2.3, HB24, and HB9. Initial sequencing and expression studies with HB9 and HB24 have been completed. Human genomic clones for HB9 and HB24 have been isolated and partially sequenced. A mouse genomic clone for HB9 has been isolated and will be used for homologous recombination experiments. Screening of a mouse B cell cDNA library with the human HB9 and HB24 cDNAs resulted in the isolation of 3 cDNA clones which are related to either HB9 or HB24, but each identifies a distinct gene. The overexpression of HB24 in Jurkat cells resulted in the induction of an activation phenotype. Besides activated lymphocytes HB9 and HB24 are expressed in bone marrow progenitor cells and differentiation along specific lineages results in disappearance of HB24 and HB9 mRNA. Studies with the human HB9 and HB24 cDNA probes have revealed expression of murine and Drosophila homologs during embryogenesis. We have completed studies examining the effects of inhibiting certain specific phosphatases in lymphocytes on the expression of the transcription factors AP-1 and NF- κ B; and the cytokine TNF- α . Studies with the vasoactive peptide endothelin have shown the presence of endothelin receptors on mast cells in addition to the production of endothelin by these cells. The production of endothelin by astrocytes is partially regulated by endothelin itself since it can induce peptide secretion.

Others:	E. Kozlow	Medical Staff Fellow	LIR, NIAID
	P. Rieckmann	Guest Researcher	LIR, NIAID
	C. Thevenin	Guest Researcher	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00431-07 LIR

PERIOD COVERED

October 1, 1990 to september 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

A Molecular Biologic Approach to Immune Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI	U. Siebenlist	Expert	LIR, NIAID
Others	J. Wright	Guest Researcher	LIR, NIAID
	V. Bours	Guest Researcher	LIR, NIAID
	K. Brown	Visiting Associate	LIR, NIAID
	P. Bressler	Senior Staff Fellow	LIR, NIAID
	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS (if any)

LP/NCI (Kelly); Squibb Institute of Molecular Biology (Bravo)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

6

PROFESSIONAL

4

OTHER

2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For several years, we have been studying in molecular detail the primary genetic response of human peripheral blood T cells to mitogenic activation. By employing subtractive cloning technology, we have previously isolated in excess of 60 genes whose expression is induced within hours of cellular stimulation. Included among these genes are well-known lymphokines, receptors and oncogenes in addition to many novel genes whose functions we are investigating. To date we have discovered a number of genes important for proliferation and the expression of a differentiated T cell phenotype; these include two novel lymphokines, three zinc finger proteins, factors which bind DNA, a steroid receptor protein, a novel phosphatase, a ras oncogene-related protein, a transmembrane receptor closely related to a receptor involved in signalling during cell killing by natural killer cells and two genes which belong to a new family of NF-κB transcription factors. We have pursued both functional as well as regulatory aspects of several selected novel genes. One of the zinc finger genes, 225, is deregulated in its expression by transfection with the human T leukemia virus, suggesting a potential function of this protein in cellular transformation. Drosophila genes sharing a similar zinc finger domain have been cloned to understand function in a genetically more easily manageable system. NF-κB complexes regulate expression of many inducible genes including most lymphokines/cytokines as well as many viruses like human immunodeficiency virus and cytomegalovirus. We have demonstrated that these many pleiotropic effects are likely to be mediated by several distinct factors including one made up of relB and rik, two novel mitogen inducible rel-related proteins distinct from any previously described constituents of NF-κB. These two factors heterodimerize and bind to κB sites and transactivate such sites in cells *in vivo*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00213-11 LIR	
PERIOD COVERED October 1, 1990 to September 30, 1991			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical, Immunopathogenic, and Therapeutic Studies in the Vasculitides and Other Immune-Mediated Diseases			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	G.S. Hoffman	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	R.Y. Leavitt	Senior Investigator	LIR, NIAID
	G.S. Kerr	Visiting Associate	LIR, NIAID
	M.C. Cid	Guest Researcher	LIR, NIAID
COOPERATING UNITS (if any) NCI, NIH, (W.D. Travis); LDBA, NIH, (H. Kleinman); LDBA, NIH, (D. Grant); NIAID, NIH, (C. Hallahan); DMID, NIH, (M.F. Cotch); DMID, NIH, (R.A. Kaslow).			
LAB/BRANCH Laboratory of Immunoregulation			
SECTION Immunopathogenesis Section			
INSTITUTE AND LOCATION National Institute of Allergy and Infectious Diseases			
TOTAL MAN-YEARS		PROFESSIONAL	OTHER
3		3	0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have established a computerized data bank to store, retrieve and analyze an extensive collection (24 years/ >200 patients) of epidemiological, clinical and laboratory information about the systemic vasculitides. We have continued to study the <u>efficacy of daily low dose cyclophosphamide (CP)</u> and prednisone as remission-inducing and potentially curative therapy in <u>systemic vasculitis</u> . The long-term toxicity of daily CP therapy in some patients has led to studies of <u>alternative treatment</u> including (1) intermittent intravenous high dose CP, and (2) weekly low dose methotrexate and (3) daily trimethoprim/sulfamethoxazole in limited Wegener's granulomatosis (WG). A prospective analysis of <u>bronchoalveolar lavage in WG</u> and controls has demonstrated that disease exacerbations are characterized by <u>neutrophilic alveolitis</u> , macrophage-leukocyte phagocytosis and the <u>production of anti-neutrophil cytoplasmic antibodies (ANCA) in the lung</u> . These observations suggest that neutrophilic inflammation and abnormal immune reactivity to neutrophil antigens may play a role in the pathogenesis of this disease. The <u>pathogenesis of the vasculitides</u> is also being investigated in vitro with the aid of <u>endothelial cell systems</u> to (1) characterize leukocyte binding to normal control and autologous endothelial cells, (2) assess antibody reactivity and serum cytotoxicity to normal and autologous endothelial cells, (3) determine whether sex hormones influence leukocyte-endothelial cell adhesion, a question that is particularly relevant to Takayasu's arteritis, and 4) assess the role of haptoglobin in stimulating angiogenesis. An <u>epidemiologic analysis of patients with WG</u> has continued to evaluate environmental factors, such as inhalant exposures and patient clusters.			

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathogenesis of *Chlamydia trachomatis* infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
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COOPERATING UNITS (if any)

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| <input checked="" type="checkbox"/> (a) Human subjects | <input checked="" type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the United States with an estimated 5 million cases annually. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop improved diagnostic assays and to examine the pathogenesis of chlamydial infection in experimental animal models. Chlamydia infections tend to have the characteristics of a prevalent infection since infection rates vary little by either sex, number of sex partners or the patient's relationship with their sexual partners. Chlamydial cervical infections in women were higher in contacts (26%) than in non-contacts (16%), particularly in contacts of men with gonorrhea (32%), suggesting that co-infection with *N. gonorrhoeae* and *C. trachomatis* are quite frequent in this population. Detection of chlamydia species by polymerase chain reaction (PCR) and genotyping by restriction enzyme analysis have been developed. In addition to differentiation of *C. pneumoniae*, *C. trachomatis* and *C. psittaci*, we have demonstrated that nearly three-quarters of *C. trachomatis* serovars are comprised of D, E and F. Serovar distribution did not correlate with clinical presentation, concurrent gonococcal infection or concordance among sexual couples. In a field study of trachoma in Tanzania, PCR detection of chlamydia genome was demonstrated to have a threefold increase in sensitivity compared to DFA, and a semi-quantitative PCR technique was utilized to monitor response to therapy in over 200 children with trachoma. In an experimental animal model where viability of *C. trachomatis* can be monitored, we demonstrated persistence of chlamydial DNA by PCR in monkeys who have become culture negative. This persistence of chlamydial DNA may possibly contribute to either a sustained hypersensitivity response or a latent state which is inhibited by the presence of neutralizing antibody and cell - mediated immunity. Finally, the 16s rRNA of *C. pneumoniae* was sequenced and compared to *C. psittaci* and *C. trachomatis*, demonstrating that *C. pneumoniae* is more closely related to *C. psittaci* than *C. trachomatis*. Additional studies utilizing primer sets from the 16s rRNA sequence are planned for PCR detection of *C. pneumoniae* as an etiologic agent of pneumonia in pediatric, adult and geriatric populations.

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Summary Statement
LABORATORY OF INFECTIOUS DISEASES
National Institute of Allergy and Infectious Diseases
October 1, 1990 to September 30, 1991

DENGUE VIRUSES

Extensive dengue epidemics caused by one or more of the four dengue virus serotypes of the flavivirus family continue to be a major public health problem in many tropical and subtropical areas. Dengue viruses have the widest geographical distribution and the highest incidence of infection among the flaviviruses, a large family of insect borne viruses containing more than 66 members. Only the US and Canada in our hemisphere are free of endemic or epidemic dengue virus infection. During the past decade Cuba, Brazil and Venezuela have suffered extensive epidemics involving millions of cases.

During the past year scientists in MVBS, LID achieved two major milestones in a dengue virus research program that is now in its seventh year. Initially, the complete viral positive strand RNA genome of dengue virus type 4 was cloned and sequenced. The viral genome was shown to have a single long open reading frame that encoded a viral polyprotein containing the three structural proteins, C, pre-M and E at the 5' terminal position followed by 7 non-structural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5. Concurrently, the strategy used by the virus to process the viral polyprotein into its constituent proteins was defined in considerable detail including the identification and characterization of cleavage sites and the cellular and viral proteases responsible for specific cleavages. In addition, individual genes or combinations of genes were expressed by a vaccinia virus or baculovirus recombinant. In this manner the viral envelope glycoprotein (E), pre membrane protein (pre M) and non-structural protein one (NS1) were identified as independent protective antigens.

Although infection with a vaccinia virus recombinant expressing dengue pre M, E and NS1 or inoculation of baculovirus-expressed pre M, E and NS1 or E alone induced complete resistance to lethal challenge in mice, these animals developed little if any E antibodies in response to immunization. Similar observations were made in monkeys immunized by inoculation with baculovirus-expressed pre-M, E and NS1 or E alone; partial resistance was induced but the E antibody response to immunization was very weak or undetectable. This suggested that immunogenicity of E (expressed by vaccinia virus or baculovirus) was suboptimal and that this may have been the limiting factor in protective efficacy in the monkey study.

The *first recent milestone* involves the development of a successful strategy to increase the immunogenicity of E. Although E is present on the surface of dengue virus, it is not displayed on the surface of cells infected by dengue virus. It was hypothesized that retention of E within the infected cell might contribute to its weak immunogenicity. To investigate this possibility, the C terminus of the E gene was deleted systematically at various sites creating C terminally truncated E proteins of varying length. These proteins were expressed in a vaccinia virus recombinant and evaluated for their: (i) localization within infected cells, (ii) display on the surface of infected cells, and (iii) immunogenicity. The results were most gratifying because deletion of the C terminal 20% of E altered its transport in the infected cell resulting in its display on the cell surface and release into the extracellular environment. Marked increase in immunogenicity was also noted and this was associated with an increase in protective efficacy. Mice immunized with a vaccinia virus recombinant expressing 100% E developed encephalitis

following intracerebral virus challenge but most animals survived. In contrast, mice immunized with a vaccinia virus recombinant expressing 80% E survived challenge without becoming ill. Further analysis indicated that cell surface localization and increased immunogenicity were properties unique to 80% E that were not observed for longer or shorter C terminally deleted E mutant proteins. These unusual and highly specific properties of 80% E appear to be due to the unmasking of an internal hydrophobic anchor domain whose C terminus is located at the site of deletion. Analogous cryptic hydrophobic anchor domains are conserved among other flaviviruses. Significantly, vaccinia virus recombinants expressing 80% E of dengue type 2 virus or Japanese B encephalitis virus were also shown to exhibit increased immunogenicity and protective efficacy. These observations suggest that deletion of the C terminal 20% of E may represent a general strategy for increasing the efficacy of flavivirus vaccines.

The *second recent milestone* represents the realization of a major goal of the MVBS, LID dengue virus research program. This goal, in a sense the Holy Grail of our initial program, was the construction of a full-length dengue virus cDNA that could serve as a template for full-length RNA transcripts that are infectious for susceptible cells in culture. After 4 years of intensive effort a functional full-length dengue virus cDNA was produced and full-length RNA transcripts of this template were shown to be infectious for cells in culture. As a consequence, it became possible for the first time to introduce mutations into full-length dengue cDNA by site-directed mutagenesis and subsequently recover viable dengue virus bearing these mutations. Viral mutants produced in this manner have proved useful for identifying regulatory elements involved in transcription, replication and packaging of viral RNA and elucidating of the mechanisms involved in processing the long viral polyprotein into its discrete proteins.

Another immediate application of full-length, functional dengue cDNA is in the area of vaccine development. Restriction of dengue virus replication should cause attenuation, however, it is essential that a proper balance between attenuation and immunogenicity be achieved so that an immunizing infection can occur without the development of undesirable signs or symptoms of disease. The identification of dengue virus mutants with this desirable balance of attenuation and immunogenicity is an empirical process and may require the evaluation of a large number of mutants. Fortunately, scientists in MVBS, LID have already constructed a panel of viable, site-specific mutants that are restricted in virus replication as a consequence of amino acid substitution in the viral polyprotein near the cleavage site between dengue virus non-structural proteins NS1 and NS2a. Certain amino acid substitutions in this region decrease the efficiency of cleavage of NS1 and NS2a thereby decreasing the overall processing of the viral polyprotein. Similarly, it has been possible to create viable deletions in the 3' non-coding region of the viral genome that restrict virus replication. Such deletion mutants offer the theoretical advantage of being less subject to reversion of phenotype than amino acid substitution mutants.

The most rational approach to the identification of dengue virus mutants which exhibit the desired balance of attenuation and immunogenicity is to screen a variety of viable mutants (with various types of mutations in various regions of the viral genome) in monkeys. These animals are best suited for this purpose because they appear to be the most satisfactory experimental surrogate for human dengue virus infection.

ROTAVIRUSES

Development and Evaluation of a Live Attenuated Virus Vaccine for Prevention of Rotavirus Disease. The "Jennerian" approach to immunization is the oldest strategy for vaccination against infectious diseases. This strategy, initially used by Jenner for immunization against smallpox 200 years ago, involves the use of a live virus derived from a non-human host to prevent human disease caused by an antigenically related virus. In addition to being antigenically related to the human viral pathogen, the animal virus surrogate must be attenuated for humans and thus suitable for use as a human vaccine. We have used a simian rotavirus derived from rhesus monkeys (RRV) as the surrogate strain for immunization against rotavirus disease in infants and small children. This virus is closely related antigenically to serotype 3 human rotavirus and is attenuated in young infants.

The "Jennerian" approach to immunization against rotavirus diarrhea has had variable success. Impressive resistance was documented in some studies but not in others. Significant protection was observed when the serotype 3 rhesus rotavirus (RRV) vaccine was used to immunize young infants who later were exposed to human rotavirus strains of the same serotype in the community. However, in other trials in which serotype 1 rotaviruses predominated in the community, vaccine efficacy was variable. Initially, it appeared that failure to induce heterotypic immunity was the explanation for failure of live rhesus rotavirus (serotype 3) vaccine to provide protection against virulent rotaviruses of other serotype, i.e., serotype 1, as well as 2 or 4. For this reason we subsequently altered our strategy to pursue a modified "Jennerian" approach utilizing single gene reassortant viruses that contained the human rotavirus VP7 gene which confers antigenic specificity of serotype 1, 2 or 4, while the remaining 10 genes were of RRV origin. These three reassortants were initially tested separately and later combined together with RRV (serotype 3) to constitute a quadrivalent vaccine. However, our analysis of clinical trials of the individual reassortant viruses indicated that the situation was more complex than initially appreciated because heterotypic resistance was documented in 4 separate studies. In other instances homotypic resistance was not observed.

In an attempt to clarify these discordant observations sera of protected vaccinees and "vaccine failures" were analyzed for their VP7 epitope-specific antibody responses to vaccination. In addition, rotavirus isolates from "vaccine failures" were sequenced in the appropriate regions of the gene that encodes the outer capsid protein VP7. These studies identified: (i) antigenic divergence between vaccine virus and naturally circulating rotaviruses and (ii) marginal immunogenicity as independent causes of "vaccine failure". The latter problem has been partially solved by increasing the dose of vaccine virus fed young infants. However, the former problem has proven more difficult to address because extensive sequence analysis and epitope mapping of many additional isolates are required. Sporadic, unanticipated protection against heterotypic virus strains also remains to be explained. More detailed epitope mapping of heterotypic rotavirus strains is currently underway so that antibody and T cell responses to epitopes shared among strains of diverse serotype can be identified and analyzed.

The laboratory support for many phase 1 and phase 2 clinical trials continues to be provided by the Epidemiology Section. This support includes: examination of stool specimens for evidence of virus shedding by vaccinees, sequence and serotype analysis of rotaviruses recovered from clinical trials, and examination of sera for antibody responses to a variety of rotavirus antigens and their epitopes. These activities provide essential data for evaluation of the safety and efficacy of candidate vaccines as well as elucidation of the natural history of rotavirus infection. Two thousand and nine vaccinees and placebo recipients were under

surveillance during rotavirus vaccine trials recently completed. At present, approximately 8000 infants and children are under surveillance, or will soon be under surveillance, in a series of collaborative vaccine trials in 5 different countries.

Alternate Strategy for Selection of Attenuated Mutants of Human Rotavirus. Three human rotavirus isolates and two human rotavirus reassortants were successfully adapted to grow in primary African green monkey kidney cells at the suboptimal temperature of 30°C, 28°C, or 26°C. These rotavirus strains were passaged in this manner in an attempt to select cold-adapted (*ca*) mutants that are attenuated and suitable for use in a live virus vaccine. Analysis of the efficiency of plaque formation of the 26°C cold-adapted serotype 1 (D) strain at various temperatures demonstrated that this virus is indeed a *ca* mutant; it produced plaques at 26°C, whereas its parent did not. In addition, this mutant is also temperature sensitive (*ts*) having a shut-off temperature for plaque formation of 37°C.

It was recently shown that chimpanzees are susceptible to human rotavirus and develop extensive diarrhea during experimental infection. As a consequence, the rapid development of a live *ca* rotavirus vaccine is now possible utilizing susceptible chimpanzees to evaluate attenuation, immunogenicity, and protective efficacy.

Immunological Correlates of Resistance to Rotavirus Infection. More extensive analysis of sera from adult volunteers who were challenged with a virulent human serotype 1 rotavirus indicated that resistance to rotavirus infection and diarrhea was strongly associated with antibodies to a serotype 1 or serotype 3 neutralization epitope that maps to amino acid 94 of the outer capsid protein VP7. This amino acid had been identified as a key residue in the formation of a major serotype-specific antigenic site during previous sequence analysis of neutralization-resistant mutants selected in the presence of VP7 serotype-specific monoclonal antibodies. Our current findings imply that antibodies directed against or near this site are associated with resistance to illness. Thus, this epitope appears to be involved in the formation of a major protective antigenic site. This information is being applied to the analysis of rotavirus vaccine studies to determine whether antibody specific for this epitope is an accurate predictor of vaccine-induced resistance. In addition, previous studies indicated that variation occurred among rotavirus field strains at VP7 amino acid residue 94. Analysis of the sequence of rotavirus strains isolated from vaccine failures is currently underway to determine whether antigenic variation at this site allows circulating rotavirus strains to escape from vaccine-induced immunity.

Analysis of the serologic response of the "vaccine failures" in a recent vaccine field trial confirmed observations from other vaccine efficacy studies. During immunization most "vaccine failures" failed to develop antibodies to: (i) the vaccine virus (as measured by IgA ELISA or by neutralization) or (ii) the serotype-specific VP7 antigenic site of the rotavirus strain that caused illness (as measured by epitope blocking assay). Thus, lack of VP7-specific serum antibody was confirmed as being a major correlate of susceptibility to illness in young rotavirus vaccinees.

INFLUENZA A VIRUSES

Intragenic Suppression of a Temperature Sensitive (*ts*) Phenotype Specified by a 12 Amino Acid Deletion in the NS Gene. The influenza A/Alaska/77 (H3N2) virus mutant 143-1 is temperature sensitive (*ts*) due to a spontaneous in-frame 36 nucleotide deletion in the non-structural (NS) gene, which created a 12 amino acid deletion in the NS1 protein. Unexpectedly, phenotypically revertant (i.e., *ts*⁺) virus was isolated readily following replication of the 143-1 virus either *in vitro* and *in vivo*. Segregational analysis indicated that an intragenic suppressor mutation was responsible for loss of the *ts* phenotype. Valine was substituted for alanine at amino acid 23 of the NS1 protein in each of the *ts*⁺ "revertants" analyzed. These findings have implications for vaccine development because a point mutation was shown to abrogate an attenuation phenotype that was specified by a significant deletion mutation. Thus, the use of deletion mutation to achieve attenuation does not insure the genetic stability of the resulting mutant virus *in vivo*.

First Successful Attenuation of Influenza A Virus by Genetic Engineering. A collaborative study was initiated between Dr. Peter Palese's laboratory (Mt. Sinai School of Medicine) and LID shortly after our New York colleagues described the first successful rescue of an influenza A virus gene from a full-length cDNA copy of the gene. This was accomplished by recombinant DNA techniques, transfection of susceptible cells with full-length RNA transcripts of full-length cDNA, gene rescue and gene reassortment. As a consequence, it is now possible for the first time to introduce specific mutations into full-length cDNA of an influenza virus gene and recover viable mutants which can be evaluated for their usefulness in immunoprophylaxis. This important advance in molecular virology and genetics was almost immediately applied to the construction of live influenza virus candidate vaccine strains. The first recombinant DNA-produced mutant to emerge from our collaboration was a resounding success.

The approach that was taken was suggested by the observation that influenza A and influenza B viruses do not undergo gene reassortment during dual infection. This restriction is thought to reflect differences in the 3' and 5' terminal non-coding sequences of influenza A and influenza B genes, domains that contain cis acting signals for viral polymerase recognition and packaging of viral RNAs into virus particles. Hence, we reasoned that a chimeric gene containing the regulatory elements of influenza B viral genes would not function efficiently in an influenza A virus and this should restrict virus replication resulting in attenuation. A chimeric intertypic neuraminidase (NA) gene that contained the coding region of the influenza A/WSN/33 virus NA and the flanking 3' and 5' non-coding sequences of the nonstructural (NS) gene of the influenza B/Lee virus was produced by recombinant DNA techniques. The chimeric gene was then introduced into the genome of influenza A/WSN/33 virus by: (i) transfection of full-length RNA transcripts of the chimeric cDNA, (ii) gene rescue during infection with the influenza A virus, and (iii) subsequent gene reassortment. The resulting chimeric reassortant virus replicated poorly in the upper respiratory tract of mice, but prior infection with this highly attenuated virus protected mice from lethal influenza A/WSN/33 virus challenge. This novel influenza A virus bearing the chimeric NA gene represents the first negative strand RNA virus that has been attenuated by introduction of specific mutations into viral cDNA. Furthermore, the properties of this engineered virus suggest that a similar strategy will probably yield other attenuated mutants of influenza virus that are safe and effective for prevention of influenza in humans.

RESPIRATORY SYNCYTIAL VIRUS (RSV)

Processing of RSV Glycoproteins. In previous work, it was shown that RSV encodes three transmembrane glycoproteins, the fusion F glycoprotein involved in viral penetration, the attachment G glycoprotein, and the small hydrophobic SH protein of unknown function. The post-translational processing of the three proteins has been studied in some detail. Inhibitors of exocytosis were employed to identify intermediates in processing and to operationally define the intracellular sites of processing steps such as oligomerization, palmitylation, polylactosaminylation, cleavage of the F protein and O-glycosylation of the G protein. Sucrose gradient sedimentation and chemical cross-linking were used to monitor oligomerization, and lectin-binding and endoglycosidases were used to monitor O-glycosylation. During recent studies it was observed that the oligomerization of the G protein occurs in the endoplasmic reticulum, whereas its O-glycosylation does not occur until the trans Golgi compartment. This implies that the O-linked sugars are not important determinants of oligomerization and, by implication, of polypeptide folding. Studies are continuing to analyze the multiple forms and complex processing scheme of the SH protein using site-directed mutagenesis and biochemical techniques.

Protective Antigens. A study was performed in mice to identify the total repertoire of protective antigens. Mice were immunized with one of a series of vaccinia-RSV recombinant viruses that separately encode a single RSV protein. Of the 9 RSV proteins studied (excluding only the L or large polymerase protein) only the F, G, N, and M2 proteins induced resistance to RSV infection. Complete or almost resistance was induced by viral surface glycoprotein F or G and this resistance was in evidence both early (9 days) and late (28 days) after immunization. In contrast, resistance induced by internal proteins N and M2 was of lesser magnitude and was relatively short lived. These observations suggest that RSV vaccines need only contain F and G glycoproteins, because the other RSV proteins either fail to induce resistance or induce immunity that is less effective and more transient than the resistance provided by the F and G antigens. The resistance induced by M2 was shown to be mediated by CD8 T-cells which explains its transient nature. Others have shown that N is also a target for CD8 CTLs.

Structural Analysis of RSV Genome and Construction of Synthetic RSV vRNAs Which Exhibit Biological Activity. One of the major goals of LID is to develop methods for producing live RSV from cloned cDNA. The ability to directly introduce changes into cDNA and thus into virus, would have important applications to molecular studies and to the production of defined attenuated vaccine strains. However, the development of this capability is complicated by the fact that RSV is a negative strand RNA virus. The viral RNA (vRNA) of such viruses is noninfectious, in contrast to the situation with DNA viruses such as adenovirus or positive-sense RNA viruses such as poliovirus and dengue virus. Instead, the minimum unit of infectivity for RSV is, by analogy to other better-known negative strand RNA viruses, a viral nucleocapsid which is competent for transcription. Thus, the production of RSV from cDNA would require transcription of synthetic vRNA from cDNA in the presence of the appropriate RSV proteins such that the vRNA is assembled into nucleocapsids.

The first step in constructing a full-length functional genome from full-length cDNA involves identification of the RSV sequences that direct transcription and replication of vRNA. During FY1991 the sequence analysis of the 15,222 nucleotide RSV vRNA was completed. Advantage was taken of this information to synthesize the 5' and 3' termini of the genome and use these sequences, that are thought to direct transcription and replication of vRNA, to

construct "mini" vRNAs. In essence, these small RNAs contain a large internal deletion which eliminates all of the viral open reading frames and has in their place a single marker gene such as the CAT open reading frame. The CAT sequence is flanked: (i) at its 3' end by the 3' vRNA terminal non-coding leader region and the consensus gene transcription - start signal and (ii) at its 5' end by the consensus gene transcription - stop/polyadenylation signal and the 5' terminal non-coding trailer region. In initial experiments, transfection of such a synthetic vRNA analog into permissive cells resulted in the expression of CAT that was dependent upon superinfection of the cells with RSV. The success of this strategy provides a system for: (i) identifying and characterizing cis-acting control sequences and (ii) identifying viral proteins that function in RNA transcription and replication. Subsequently it should be possible to use this information to rescue live RSV from full-length RNA transcripts prepared from a full-length 15,222 nucleotide RSV cDNA that is currently under construction. The benefits of such an RSV rescue system will be manifold because it will allow us to introduce specific mutations into RSV for molecular studies as well as for the production of a new generation of effective, more stable live attenuated vaccine strains.

RSV: Potentiation of Disease by Prior Immunization With Viral Subunits. During studies conducted in the 1960's, children previously immunized with a formalin-inactivated RSV vaccine (FI-RSV) developed more severe pulmonary disease during subsequent natural infection with RSV than did control subjects. Previously, it was shown that cotton rats immunized with FI-RSV or immunoaffinity-purified F glycoprotein also developed enhanced pulmonary histopathology following subsequent intranasal challenge with RSV. This phenomenon was studied in greater detail during the past year.

Cotton rats previously immunized with a purified RSV chimeric FG glycoprotein also developed enhanced pulmonary histopathology following challenge with RSV. This potentiation of pathology was not observed in animals previously infected with RSV or previously immunized with an adenovirus-F or vaccinia-F recombinant. Unlike RSV infection or infection by an adenovirus-F or vaccinia virus-F recombinant, which each induced a moderate to high level of serum RSV neutralizing antibodies, immunization with RSV FG induced a low titer of serum RSV neutralizing antibodies, although the total quantity of antibodies produced was very high. As a consequence, FG immunized cotton rats were not resistant to RSV challenge. These abundant "low quality" antibodies do not appear to be responsible for enhanced pulmonary pathology during subsequent RSV infection because passive transfer of post-immunization sera to other animals did not result in enhancement of lung lesions during RSV infection. However, these antibodies fail to protect the host and thus play a critical role in the development of disease potentiation. These observations suggested that enhanced histopathology is mediated by RSV-specific T-cells. This predication was shown to be correct during subsequent studies in mice.

Enhanced pulmonary histopathology during RSV infection was also observed in mice previously immunized with formalin-inactivated (FI) vaccine. FI RSV-immunized mice depleted of CD4 T-cells prior to RSV challenge, failed to develop enhanced pulmonary histopathology indicating that CD4 T-cells were the mediators of potentiation. On the other hand, depletion of CD8 T-cells did not affect potentiation.

Development and Evaluation of Candidate Live Attenuated RSV Vaccine Strains. The realization that RSV subunit glycoprotein preparations such as F or a chimeric F-G, also caused the potentiation of pulmonary histopathology previously observed with formalin-inactivated whole RSV vaccine, stimulated us to re-examine live attenuated RSV vaccine candidates

developed 10 to 20 years ago. The 26°C cold passaged mutant of RSV was not pursued because of minor clinical reactions, whereas the *ts*-1 mutant and its re-mutagenized derivatives, *ts*-1 NG-1 and *ts*-1 NG-16, were not studied further because of instability of the *ts* phenotype during infection of susceptible infants or chimpanzees. In retrospect, our decision 10 years ago to terminate the study of *ts* mutants of RSV may not have been warranted. At that time, it was not known that live poliovirus vaccine strains undergo mutation with very high frequency without causing disease. For example, one of the two major mutations that contribute to the greatly reduced neurovirulence of the type 3 vaccine strain for monkeys, a base substitution at position 472 in the 5' noncoding region of the viral genome, regularly reverts to wild type sequence early during infection of the gastrointestinal tract, but the risk of paralytic disease during immunization is extremely low, i.e., $\sim 10^{-6}$. A similar restoration of wild type sequence at the site of the corresponding mutation in type 1 poliovirus vaccine strain also occurs in $\sim 50\%$ of immunizations. These observations suggest that it is not wise to set an unrealistic goal of complete genetic stability and reject candidate vaccine strains that exhibit any instability without first determining the clinical significance and acceptability of genetic changes that occur during immunization. For these reasons we have reinitiated studies with the *ts* and *ca* mutants. Currently we are exploring two basic approaches for producing live attenuated viruses, namely selection and characterization of cold-adapted (*ca*) or temperature-sensitive (*ts*) mutants. Four RSV mutants were previously identified that were either: (i) highly attenuated in susceptible infants, i.e., *ca* RSV and *ts*-1 RSV, or (ii) more defective and more stable with regard to *ts* phenotype than their highly attenuated *ts*-1 parent, i.e., the further mutagenized *ts*-1 NG-1 and *ts*-1 NG-16 derivatives of *ts*-1. The first two mutants, i.e., *ca* RSV and *ts*-1 RSV, represent excellent candidates for the introduction of additional mutations because both viruses are highly attenuated for susceptible infants and require only the loss of a low level of residual virulence to yield mutants which exhibit the desired balance of satisfactory attenuation and protective immunogenicity. Currently, the 26° *ca* mutant is being adapted to growth at a lower temperature in an attempt to select for additional attenuating mutations that do not compromise immunogenicity. In addition, *ts* mutations are being introduced into the *ca* mutant. The third and fourth mutants (*ts*-1 NG-1 and *ts*-1 NG-16) may already possess this desired balance, but studies in chimpanzees and clinical evaluation in susceptible infants are required to make this determination. In any case, if *ts*-1 NG-1 and/or *ts*-1 NG-16 do retain a low level of residual virulence, additional mutations, such as *ca* mutations, can be introduced in an attempt to achieve satisfactory attenuation with retention of protective efficacy. Also, subgroup B *ca* and *ts* viruses are being generated.

PARAINFLUENZA VIRUS TYPE 3 (PIV3)

Parainfluenza virus type 3 (PIV3), which causes severe lower respiratory disease in infants and young children, is second only to RSV as cause of viral respiratory disease requiring hospitalization. For these reasons a vaccine to prevent this illness is needed. Significant progress has been made over the past year in the development and evaluation of a candidate live attenuated PIV3 vaccine. Cold-adapted mutants of PIV3 at cold-passage (cp) 12, 18 or 45 were evaluated in rhesus monkeys and humans. The cp18 virus retains mild virulence for seronegative infants and is able to spread to contacts. Therefore it is not acceptable for use as a vaccine. The cp45 virus is significantly more attenuated than cp18 and has been shown to contain both non-*ts* and *ts* attenuating mutations. This mutant appears to be stable because it retains its *ts* phenotype after replication in monkeys. Phase 1 studies in humans have progressed satisfactorily and currently, the cp45 virus is being evaluated in seronegative infants and in chimpanzees. At present, the cp45 *ca* mutant appears to be a promising candidate live

virus vaccine strain. In addition, the "Jennerian approach" to immunization shows promise. This approach has yielded a second candidate vaccine virus, namely bovine PIV3. This virus is currently being evaluated in seronegative infants. Two of 4 infants administered 10^3 TCID₅₀ of the bovine PIV3 strain shed the virus but did not develop any symptoms.

The parent PIV3 (strain JS) from which the cp45 virus was derived, has been completely sequenced and a full length clone is being constructed for *in vitro* rescue studies as detailed for RSV in the preceding section.

HEPATITIS VIRUSES

New Hepatitis Viruses. In 1989 a possible paramyxovirus etiology for giant cell hepatitis was proposed, based on electron micrographic changes seen in the liver of patients with this disease. Collaborative studies, including attempts to transmit the disease to primates, are in progress. Patients with thalassemia in Sardinia, Italy, receive monthly blood transfusions as therapy. Many of these patients have developed chronic hepatitis. Most cases could be identified as hepatitis B or hepatitis C, but some lacked markers of either of these viruses. These patients are being studied for evidence of previously unrecognized hepatitis viruses.

Hepatitis A Virus (HAV) - Genetic Determinants of Growth in Cell Culture. Hepatitis A virus (HAV) is a picornavirus with a single-strand, positive sense RNA genome of approximately 7500 nucleotides. Wild-type HAV grows poorly in cell-culture, and is not cytopathic. A cell-culture adapted mutant has been isolated which grows significantly more efficiently in cell-culture. This mutant is attenuated for marmosets and chimpanzees. The question of mutations responsible for efficient growth in cell culture was addressed by: (i) constructing full-length chimeric cDNAs containing a portion of the wild-type virus genome and the remainder derived from the cell culture-adapted mutant and (ii) analyzing the ability of full-length chimeric RNA transcripts to initiate efficient infection *in vitro*. These experiments demonstrated that mutations in the non-structural protein 2BC region of the HAV genome were responsible for adaptation to efficient growth *in vitro*, while mutations in the 5' noncoding region imparted a cell tropism phenotype. Additional chimeric viruses were produced by oligonucleotide-directed mutagenesis of the cDNA clones and analysis of these clones indicated that three of the six mutations in the 2BC region had no effect on growth *in vitro*, while any two of the remaining three mutations were able to enhance growth *in vitro* significantly. However, the full complement of these three mutations was required for optimal growth of HAV in cell culture. Currently, a similar strategy is being used to identify the site of mutations that confer attenuation on the tissue culture adapted mutant. Adaptation to efficient growth in cell culture probably includes a change in cell tropism in which a mutant is selected that will grow well in a cell type, such as monkey kidney or lung fibroblast, that is not a component of HAV's normal cell repertoire *in vivo*. Attenuation would be anticipated as a consequence of the failure of HAV to replicate efficiently in cells normally involved in pathogenesis of disease, however, it is not clear at this time if the tissue culture adaptation mutations play a significant role in attenuation.

Simian HAV - Possible Use in "Jennerian" Immunization. An HAV recovered in Moscow from an African green monkey with hepatitis was analyzed for its sequence divergence from HAV strains recovered from humans. This strain appears to be the most divergent HAV studied thus far. The nucleotide sequence of the simian isolate (AGM-27) differs by 20% from that of human HAV strains and another simian isolate. The difference in amino acid sequence from

the latter viruses is 10%. The host range of the AGM-27 strain is of some interest because it causes hepatitis in African green monkeys and marmosets but it is avirulent in chimpanzees. This host range pattern suggests that the AGM-27 strain may be attenuated for humans. If this proves to be the case, it may be possible to use the simian HAV for immunization of humans against HAV.

Hepatitis C Virus (HCV). Hepatitis C virus (HCV) is an enveloped positive strand RNA virus that is a major cause of transfusion related non-A, non-B (NANB) hepatitis. The sequence of a 1977 isolate of HCV and that of a more recent isolate obtained in 1980 from the same persistently infected patient were compared. Differences were observed at 123 (2.5%) of the 4,923 nucleotides sequenced, suggesting that the mutation rate of the H strain of HCV is approximately 1.9×10^{-3} base substitutions per genome site per year. The nucleotide changes were exclusively base substitutions and were unevenly distributed throughout the genome with a relatively high rate of change observed in the nonstructural protein number 1 gene. Thus, the mutation rate of the HCV genome is similar to that of other single strand RNA viruses, however, HCV genes appear to be evolving at different rates within the virus genome.

Although the development of a serologic assay to detect antibody to HCV has greatly extended our knowledge of NANB hepatitis, little is known about the course of viremia during HCV infection. For this reason, the polymerase chain reaction assay was used to define the temporal pattern of serum HCV RNA and its relationship to antibody response and clinical outcome in the course of NANB hepatitis. In the majority of patients examined, HCV RNA appeared in serum within 1 week post-infection, preceding by several months the clinical onset of hepatitis and seroconversion. Viremia was transient, lasting only a few months in acute self-limited hepatitis, but persisted up to 14 years in patients who were chronically infected. Serum HCV RNA may provide prognostic information regarding progression of acute to chronic virus infection.

Hepatitis E Virus (HEV). HEV is a major cause of large outbreaks of hepatitis associated with common source contaminated water. The incubation period is similar to HAV, i.e., ~ one month, but HEV is not related to HAV. The experimental host range for HEV is relatively broad including cynomolgus monkeys, chimpanzees, and rhesus monkeys. Pig-tailed macaques and marmosets appear to be resistant. Although sequence analysis of HEV clones is still rather limited, preliminary data indicate that this virus, like most single-strand RNA viruses, exhibits significant heterogeneity of genomic sequence. This has potential implications for vaccine development, since heterogeneity of the degree exhibited by this virus is sometimes associated with significant antigenic variation. However, the limited data available from cross-challenge studies in experimental primates suggest that even relatively divergent strains elicit cross-protective antibodies.

Hepadnaviruses: Woodchuck Virus. The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus, WHV infection of woodchucks provides a relevant and convenient model for achieving a better understanding of human HBV infection and its consequences. The degree of genome variation that exists among WHV replicative intermediates was studied by isolating supercoiled molecules from the nuclei of liver cells of a woodchuck infected with WHV and then cloning and sequencing these materials. There were a total of 5 differences among the clones including 4 nucleotide substitutions and a 73 nucleotide deletion. The recombinant WHV DNAs were then assayed for infectivity.

Transfection of monomeric, circular DNA into the liver of neonatal woodchucks demonstrated that only 1 of the 3 recombinants was infectious. Woodchucks transfected with the infectious recombinant became positive for WHV surface antigen (WHsAg) and antibody to the virus core protein (anti-WHc) at 3 months post-transfection. These data suggest that both wild type (i.e., replication competent) and mutant (i.e., replication negative) WHV replicative intermediates are present in infected woodchucks.

Next, the frequency of chronic virus infection in animals inoculated with two different WHV serum pools was compared. Neonatal woodchucks were infected with 5×10^6 WHV genome equivalents of either a WHV serum pool containing both wild type and mutant virus, or, a pool containing only wild type genomes. The chronic carrier rate of both groups was found to be the same. This suggests that defective virus is not essential for the establishment of persistent hepadnavirus infection.

SIMIAN ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

The major goal of this Project is to establish an experimental animal model for human AIDS in which to study viral determinants of immunodeficiency and immunologic correlates of resistance to infection or disease. Simian immunodeficiency virus (SIV) isolated from sooty mangabey monkeys (SIVsm) readily induces an immunodeficiency syndrome in macaques similar to human AIDS. Therefore, this strain has been used in studies of pathogenesis and experimental immunoprophylaxis.

African Simian Lentiviruses. The genetic analysis of African strains of SIV has included SIVsyk which was recovered from a sykes monkey and SIV which was isolated from the tantalus sub-species of African green monkey (AGM). Sequence analysis of the sykes isolate indicated it to be a distinct new member of the primate lentiviruses. The tantalus strain groups with other African green monkey SIVs but forms its own species-specific subgroup.

Molecular Pathology of End State SIV-induced Disease. Analysis of the molecular pathology of end-stage SIVsm-induced immunodeficiency indicated that large amounts of unintegrated viral DNA were present in most tissues but macrophages appeared to be the major reservoir of SIV. Sequence analysis of molecular clones isolated directly from tissue demonstrated that SIV, similar to other single strand RNA viruses, exhibits genomic heterogeneity, i.e., the viral genome is actually a swarm of related genomes. Each of 10 infectious clones obtained directly from the spleen of one of these macaques was distinctive with regard to sequence and cell tropism. The region responsible for the fine variation in tropism was mapped to a region of gp120 distinct from the CD4 binding region. Progeny virions of one of these clones induced a persistent decline in circulating CD4 lymphocytes within 6 months of infection. Viruses isolated from infected tissues by co-cultivation appeared to be selected from the heterogeneous SIV population by their ability to grow in cell culture and were not representative of the wide spectrum of genotypes present in tissue.

Genetic Drift of SIV *In Vivo*. Genetic drift of SIV in vivo was studied in 4 monkeys infected with a SIVsm molecular clone. Our analysis indicated that SIV undergoes significant variation upon replication in vivo and this variation is far greater in the envelope gene than in the integrase gene. Some regions of env remained constant (i.e., the CD4 binding region, and the rev responsive element). Most of the substitutions were non-synonymous, while the majority of clones from some animals demonstrated a predisposition for G to A transitions and contained

multiple, in-frame stop codons in *env*. There was a correlation between multiple in-frame stop codons in the envelope gene and outcome of infection. The 2 healthy surviving monkeys had multiple stop codons in the SIV envelope gene, whereas both of the monkeys which died lacked such mutations in the majority of the envelope sequences analyzed. Finally, novel forms of viral DNA were identified that appeared to be transcripts of subgenomic spliced RNAs, implying that all of the viral RNAs (not only those of genomic-length) are reverse transcribed.

Molecular Analysis of an Acute Lethal Variant of SIV. A variant of SIV_{sm}, designated SIV_{sm}/PBj, induces an acute illness in pigtail macaques characterized by fulminant diarrhea and death within 7 to 9 days. Full-length infectious molecular clones SIV_{sm}/PBj were generated recently. The duplicated core enhancer characteristic of SIV_{sm}/PBj did not appear to be the sole determinant of the extreme virulence of this virus as proposed by other workers. Virus stocks generated from two PBj clones (sharing a common 3' end) induced the typical PBj syndrome of acute early death. Studies utilizing these clones are now underway to define the genetic basis for the acute early death syndrome.

Development of an Effective Inactivated SIV Vaccine. Our first effort to develop an inactivated whole SIV vaccine was highly successful. Macaque monkeys immunized with psoralen-inactivated whole SIV developed a robust SIV antibody response and were protected from challenge with fifty monkey infectious doses₅₀ of cell-free virus of an heterologous divergent SIV strain as well as the homologous SIV strain used to produce the vaccine. This vaccine trial is the first to demonstrate such broad protection using a molecular clone as the vaccine virus. The vaccinated animals will be challenged with a titrated suspension of cell-associated virus within the next few months. Future studies of the psoralen-inactivated SIV_{sm} vaccine will also include attempts to augment the extent and duration of resistance induced by the vaccine. It may be possible to achieve these objectives by using the vaccine to prime animals and subsequently boost their level of resistance and the duration of their immunity by: (i) infection with a recombinant vector expressing SIV envelope or (ii) inoculation of purified SIV envelope. Alternatively, the reverse sequence may prove to be more effective.

FELINE ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

Feline immunodeficiency virus (FIV) is a member of the lentivirus subfamily of retroviruses that is associated with an immunodeficiency syndrome in infected domestic cats. FIV is distantly related genetically to the simian and human immunodeficiency viruses, however the disease with which this virus is associated has spawned considerable interest in the development of the FIV/cat system as an animal model for AIDS. Previously, LID scientists had determined the complete sequence of the prototype strain of FIV. In addition, molecular clones of this strain were derived and shown to be infectious for both tissue culture and SPF cats.

To date, experimental infection of cats with the prototype strain of FIV has produced little if any disease. Two approaches were undertaken to identify isolates of FIV or FIV-related lentiviruses that might prove useful in the development of an experimental cat model for AIDS. Strain FIV-MA, isolated from an ill two year old domestic cat in Maryland, was used to infect SPF weanling kittens. Unlike our previous experience with experimental FIV infection of SPF kittens, the FIV-MA infected animals failed to gain weight at a normal rate and at five weeks post-infection (p.i.) the animals displayed symptoms of a neurological disorder characterized by

"compulsive roaming" behavior. Preliminary neurological examination suggested that the affected kittens displayed signs of an early stage of encephalopathy.

Seven hundred and seventy-one serum or plasma samples from North American and African, free-ranging and captive felids were screened by FIV immunoblot for serological evidence of feline lentiviral infection. Cross-reactive antibodies to FIV were detected with highest frequency in puma, East and South African lion and East African cheetah populations. Novel isolates of feline lentiviruses (puma lentivirus-PLV) were obtained by PBL coculture from several free-ranging Florida panthers (*Felis concolor coryii*). Sequence comparison of the PLV-RT gene with that of FIV showed a significant degree of diversity between the two feline lentiviruses.

HONORS AND AWARDS

Robert M. Chanock, M.D.

Invited participant in Fifth Colloquium on Human Diseases, Stony Brook, October 19-20, 1990. "Mucosal virus infections: RSV, rotavirus."

Invited participant, Conference: Pathogenesis and protection studies in Animal Models, Annecy, France, October 23-25, 1990. Sponsored by Foundation Merieux. "A historical perspective on RSV vaccines."

Invited member of WHO Expert Advisory Panel on Virus Diseases.

1990 Award Recipient of ICN International Prize in Virology, awarded by ICN Pharmaceuticals, Inc.

Invited guest speaker, Fourth Annual Research Day of the Marshall University School of Medicine, April 9, 1991. "Epidemiology, pathogenesis, therapy and prevention of disease caused by respiratory syncytial virus" and "New approaches to development of new or more effective viral vaccines."

Invited speaker, Festschrift honoring Dr. Saul Krugman, April 12, 1991, New York City Medical Center. "Serious respiratory tract disease caused by respiratory syncytial virus: Prospects for improved therapy and effective immunization."

Albert Z. Kapikian, M.D.

Invited to participate in conference on Immunology of Milk and the Neonate, October 14-17, 1990. University of Alabama at Birmingham.

Invited to participate in meeting on "Anti-Diarrhoeal Vaccine Priorities", World Health Organization, March 6, 1991, Geneva.

Organizer of workshop on Rotavirus at the 17th Pacific Science Congress in Honolulu, HI, May 27-June 2, 1991.

Ching-Juh Lai, Ph.D.

Invited speaker, VIIIth International Congress of Virology, August 26-31, 1990, Berlin, Germany.

Invited speaker, Modern Approaches to New Vaccines meeting, September, 1990, Cold Spring Harbor Laboratory, NY.

Invited speaker, The 39th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 4-8, 1990, New Orleans, LA.

Invited speaker, Wallace P. Rowe Seventh Annual Symposium on Animal Virology - Viral Pathogenesis, February 4-5, 1991, Bethesda, MD.

Invited speaker, Contractors Meeting on Development and Testing of Flavivirus Vaccines. Walter Reed Army Institute of Research, March 5, 1991, Washington, DC.

Invited speaker, Meeting on International Cooperation Between Brazil and the United States to Develop a Live Attenuated Dengue Vaccine, March 22, 1991, Washington, DC.

Brian R. Murphy, M.D.

Invited to present lecture "RSV Vaccines" University of Massachusetts Medical School, Worcester, MA, October 3, 1990.

Co-organizer, Meeting on RSV: Pathogenesis and protection studies in animal models. Annecy, France, October 23-25, 1990. Sponsored by Foundation Merieux.

Invited to give seminar "Current status of live attenuated influenza vaccines" January 10-11, 1991. Department of Immunology and Medical Microbiology, University of Florida.

Invited to present lecture "Influenza Viruses" to Department of Immunology and Infectious Diseases, Johns Hopkins University, February 13, 1991.

Invited to serve as chairman for the Steering Committee on Acute Respiratory Diseases and Measles, WHO, Geneva, April 23, 1991.

Invited to serve as WHO Temporary Adviser in the 8th session of the Scientific Advisory Group of Experts (SAGE) of the Programme for Vaccine Development and Transdisease Vaccinology, WHO, Geneva, June 26, 1991.

Robert H. Purcell, M.D.

Invited speaker, "Hepatitis Viruses", Uniformed Services University of the Health Science, Bethesda, MD, January 23, 1991.

Invited speaker, "Hepatitis C", Walter Reed Army Medical Center, Washington, DC, January 31, 1991.

Invited speaker, "Hepatitis Viruses", Parts 1 and 2, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD, February 22 and 25, 1991.

Invited speaker, "Hepatitis C and E Viruses", Wallace P. Rowe Seventh Annual Symposium on Animal Virology, Bethesda, MD, February 4, 1991.

Invited speaker, "Hepatitis C, Current Concepts", Albert Einstein College of Medicine, New York City, NY.

Co-organizer and speaker, "Viral Heterogeneity", Congress on Genetic Heterogeneity of Hepatitis Viruses: Clinical Implications, Sestriere, Italy, April 5-7, 1991.

Co-organizer and chairman, US-Japan Cooperative Medical Science Program, Annual Meeting of the Hepatitis Panels, New York City, NY, May 16-17, 1991.

Recipient, Award for Achievement in Hepatitis Research, and invited speaker, "Hepatitis in the 1990's", 10th Anniversary Celebration of Japan Liver Research Foundation, Tokyo, Japan, May 20, 1991.

Peter L. Collins, Ph.D.

Editorial Board, *Journal of Virology*.

Invited speaker, VIIIth International Congress for Virology, Berlin.

Invited speaker, "Animal Models of Respiratory Syncytial Virus Infections", Annecy, France.

Invited speaker, "Transcription and Replication of Negative Strand Viruses", Madrid.

Invited speaker, University of Minnesota Medical School.

Ad Hoc grant reviewer for: (a) USDA Competitive Research Grants, (b) World Health Organization, (c) Swiss National Research Foundation.

Suzanne U. Emerson, Ph.D.

Invited to give seminar "Hepatitis A Virus: Molecular Approaches to Define Pathogenesis", January 23, 1991 at DIR/LMG/NINDS/NIH.

Invited to give seminar "Hepatitis A Virus: Molecular Approaches to Vaccine Development", February 20, 1991, at NIAID Research Grand Rounds.

Invited to give seminar "Molecular and Biological Studies of Hepatitis A Virus", April 17, 1991, for DIR/LMM/NIAID/NIH.

Invited to present "Biological and Molecular Comparisons of Human (HM-175) and Simian (AGM-277) Hepatitis A Viruses", April 7, 1991, at meeting in Sestriere, Italy on "Genetic Heterogeneity of Hepatitis Viruses: Clinical Implications."

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00459-06 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Dengue and Other Flavivirus Proteins Using Baculovirus as a Vector

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Chun-Fa Zhang, Ph.D. Special Volunteer LID, NIAID

Others: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, DC (Summers, Eckels, Dubois, Sumiyoshi)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Earlier studies demonstrated that immunization of monkeys with baculovirus-expressed dengue virus proteins C, PreM, E and NS1, or with baculovirus-expressed dengue virus E alone induced only partial protection against dengue virus infection. Analysis of sera from immunized monkeys showed that antibodies to E were low or not detectable. Thus, the poor immunogenicity of E was identified as an obstacle to further development of a subunit protein strategy using the recombinant DNA approach. Fortunately, recent studies of vaccinia virus expressed E showed that a C-terminally truncated E, approximately 80% in length, is more immunogenic and protective than the full-length E in mice. Unlike the full-length E, 80% E is expressed on the cell surface and is secreted extracellularly. These new properties of truncated E probably play a major role in its increased immunogenicity and efficacy. In any case, the increase in immunogenicity and efficacy associated with the specific truncation of E has implications for the development of effective subunit vaccines for dengue as well as other flavivirus diseases. For these reasons, we constructed a total of 8 recombinant baculoviruses that expressed full-length E or 80% E of 3 different dengue virus serotypes (type 4, 2, and 3) and Japanese B encephalitis (JE) virus. The baculovirus-expressed 80% E of dengue type 4 or type 2 virus was secreted into the medium fluid of infected cells and we are currently purifying secreted E and will evaluate its immunogenicity in mice and in primates.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00476-06 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Engineering the Genome of Dengue Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

Others: Hiroyuki Hori, M.D. Visiting Associate LID, NIAID
Michael Bray, M.D. Senior Staff Fellow LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00477-06 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Analysis of Dengue Virus Envelope Glycoprotein and its Immunogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ruhe Men, M.D. Special Volunteer LID, NIAID

Others: Michael Bray, M.D. Senior Staff Fellow LID, NIAID
Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, DC (Eckels)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1990-91

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00499-05 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Selected Dengue E Protein Sequences by Recombinant Vaccinia Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael Bray, M.D. Senior Staff Fellow LID, NIAID

Others: Lewis Markoff, M.D. Medical Officer LID, NIAID
Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

LVD, NIAID, NIH (Moss)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1990-91

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00500-05 LID												
PERIOD COVERED October 1, 1990 to September 30, 1991														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Processing and Immunogenicity of Dengue Type 4 Virus Nonstructural Protein NS1														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Barry Falgout, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">LID, NIAID</td> </tr> <tr> <td>Others:</td> <td>Ching-Juh Lai, Ph.D.</td> <td>Head, MVB Section</td> <td>LID, NIAID</td> </tr> <tr> <td></td> <td>Lewis Markoff, M.D.</td> <td>Medical Officer</td> <td>LID, NIAID</td> </tr> </table>			PI:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID	Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID		Lewis Markoff, M.D.	Medical Officer	LID, NIAID
PI:	Barry Falgout, Ph.D.	Senior Staff Fellow	LID, NIAID											
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section	LID, NIAID											
	Lewis Markoff, M.D.	Medical Officer	LID, NIAID											
COOPERATING UNITS (if any)														
LAB/BRANCH Laboratory of Infectious Diseases														
SECTION Molecular Viral Biology Section														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892														
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:												
0.5	0.5	0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We previously studied the processing of dengue virus nonstructural protein NS1 by <i>in vitro</i> transcription/translation, and by <i>in vivo</i> expression using vaccinia virus as a vector. <i>In vitro</i>, the full length NS1-NS2A precursor was made, and was translocated into dog pancreas microsomal membranes and glycosylated, but NS1/NS2A cleavage did not occur. <i>In vivo</i>, brefeldin A blocked secretion of NS1 but did not inhibit NS1/NS2A cleavage. Taken together, these results suggested that NS1/NS2A cleavage occurs in the Golgi, or in a compartment between the ER and the Golgi. We have now shown that two independent methods that block ER to Golgi transport (the drug CCCP and incubation at 14°C) block NS1/NS2A cleavage. This confirms that NS1/NS2A cleavage does not occur until after NS1-NS2A exits the ER. Previously, we had also analyzed a recombinant virus expressing chimeric 72%prM-NS1(8)-NS2A protein, containing only the 8 C-terminal residues of NS1. The chimera was cleaved at the NS1/NS2A junction, indicating that only the last 8 amino acids of NS1 are required for cleavage. More recently mutants were constructed which lacked the C-terminal 31% of NS2A or the signal sequence. Both of these changes blocked cleavage of the chimeric protein, as expected from studies of authentic NS1/NS2A cleavage. </p>														

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00501-05 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Processing of Dengue Viral Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of *in vitro* processing of the dengue virus polyprotein were continued. Such studies are justified by the following rationale: (1) Flavivirus morphogenesis occurs at the ER membrane. Therefore, this event can conceivably be studied *in vitro*. (2) The flavivirus polyprotein can be thought of as a polytopic membrane protein which is internally cleaved. Principles governing the association of the polyprotein with membranes have broader implications for understanding the assembly of proteins into membranes in general. (3) The phenotype(s) "Ess" of some mutations potentially useful for vaccine development when incorporated into infectious RNA prepared from full-length dengue cDNA can be tested *in vitro*.

Flavivirus structural proteins capsid (C), pre-membrane (prM), and envelope (E) are in that order cleaved from the amino-terminus of the flavivirus polyprotein co-translationally by the host cell enzyme, signalase. prM and E each have a hydrophobic carboxy-terminus 40 to 47 amino acids in length assymmetrically interrupted by a conserved hydrophilic residue or residues. In dengue virus type 4 cDNA, the hydrophobic carboxy-terminus of prM spans residues 243 to 279 of the polyprotein, with an interrupting Arg at position 264. It is predicted that hydrophobic residues 243 to 263 constitute a transmembrane segment for prM and that hydrophobic residues 265-279 constitute a signal for E. The requirement for the conserved interrupting hydrophilic residue is not understood. We studied the processing of prM, E, and NS1 translated from RNA transcripts prepared from dengue virus type 4 cDNA. Mutations were introduced into sequences, encoding the hydrophobic carboxy-terminus of prM, and the phenotype of those mutations were compared to that of the wild-type (wt) sequence. The results of this study permit the establishment of a model for understanding the processing of the flavivirus structural proteins and the mechanisms by which their final membrane orientation is attained.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00502-05 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Antigenic Analysis of the Dengue Virus Envelope Glycoprotein (E) Using Synthetic Peptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID Others: Robert M. Chanock, M.D. Chief LID, NIAID		
COOPERATING UNITS (if any) FDA (Berkower); Torrey Pines Institute for Molecular Studies, La Jolla, CA (Houghton)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: right;">0.4</div>	PROFESSIONAL: <div style="text-align: right;">0.3</div>	OTHER: <div style="text-align: right;">0.1</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We attempted to define the immune response to the dengue type 4, strain 814669, envelope glycoprotein (E) in Balb/c (H-2^d) and CBA/N or Balb/K (H-2^k) mice using 38 synthetic 15-amino-acid peptides that span the E sequence (previously described). An <i>in vivo</i> assay in which mice were immunized with peptide(s) followed by infectious dengue virus and an <i>in vitro</i> assay of proliferation in response to peptides of T lymphocytes isolated from virus immunized mice were conducted. Peptides 39 (amino acids 137 to 151) and 41 (158-172) elicited high titer peptide and dengue 4 virus binding antibodies in Balb/c mice, as measured by ELISA. Peptides B (30-55), 48 (279-292), 71 (199-212), and 53 (368-382) elicited IgG class peptide-binding antibodies only. Peptide 54 (381-395) appeared to contain a T cell epitope only. In CBA/N or Balb/k mice, peptide 67 (17-30) elicited a response comparable to that of peptides 39 and 41 in Balb/c mice. Peptides 48, 53, and 72 (233-246) elicited high titer IgG peptide antibodies only. The results of the <i>in vitro</i> T cell proliferation assay, using lymphocytes isolated from virus-immunized Balb/c mice, confirmed that peptides 41, 48, 71, and B contain T helper cell epitopes. Surprisingly, peptides 67 and 72 which were positive in the <i>in vivo</i> assay in CBA/N (H-2^k) mice, were also recognized <i>in vitro</i> by lymphocytes from virus-immunized Balb/c (H-2^d) mice. Lymphocytes from CBA/N mice immunized with virus appeared to recognize only peptide 48 and peptide 30 <i>in vitro</i>. Peptide 30 (1-15) represents a segment in the dengue type 4 E amino-terminal to that of peptide 67 (17-30), which gave the strongest immune response in the <i>in vivo</i> assay. Lymphocytes from peptide 67-immunized CBA/N mice did proliferate when exposed <i>in vitro</i> to peptide 67 and peptide 31 (27-40), which overlaps the carboxy-terminus of peptide 67. These results suggested that peptide 67 (and probably peptides 53 and 72) contains a potential T helper cell epitope at its carboxy-terminus recognizable by H-2^k mice but that this sequence is not presented as a normal consequence of processing of E in virus-immunized mice. These results obtained in CBA/N mice await repetition in Balb/k mice (the H-2^k strain most congenic with Balb/c). Additional data show that priming with a given peptide in the <i>in vivo</i> assay could result in an anamnestic response to linear epitopes not included on the priming peptide, shortly after the mice were given infectious dengue 4 virus intraperitoneally. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00531-04 LID</div>
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis of Dengue Nonstructural Proteins NS2B and NS3		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Barry Falgout, Ph.D.	Senior Staff Fellow LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVBS LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	0.5	PROFESSIONAL: 0.5 OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We previously showed for dengue type 4 (DEN4) that both NS2B and NS3 together constitute a protease that cleaves the NS2A/NS2B, NS2B/NS3 and NS3/NS4A junctions. Others have proposed that NS3 is a viral protease based on the finding that the N-terminal 180 amino acids of NS3 contains four regions of homology to serine proteases. The role of NS2B in the protease activity is less clear. We initiated studies to mutagenize NS2B and the protease domain of NS3 to further characterize their roles in proteolytic processing. The PTM 1/v TF7 transient expression system was employed to study proteolytic processing of NS2B-30%NS3. Efficient cleavage yielding NS2B and 30%NS3 was observed in cells transfected with recombinant DNA coding for polyprotein precursor. Seven mutants were produced by introducing a deletion into the NS2B portion of this construct. Five of these mutants cleaved as efficiently as the wild-type sequence. One mutant showed slightly reduced cleavage, while another mutant (Bgl II/Nru I) was completely defective for cleavage. Further mutagenesis in this important region should identify the residues of NS2B critical for protease activity. Expression of NS2B-30%NS3 in <i>E. coli</i> from the IPTG inducible tac promoter was lethal to cells. Incorporation of the Bgl II/Nru I mutation into this construct did not eliminate this lethality, whereas truncation of NS3 allowed growth. This observation is consistent with the notion that expression of NS3 alone, which contains the proposed protease domain, is lethal to <i>E. coli</i>. If this is the case, it should be possible to isolate mutants that are defective for the NS3 protease activity. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00557-03 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cross-Reactive Antibodies to Clotting Factors in Patients Infected with Dengue Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID		
COOPERATING UNITS (if any) WRAIR, Bangkok, Thailand (Innis)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: right;">0.2</div>	PROFESSIONAL: <div style="text-align: right;">0.2</div>	OTHER: <div style="text-align: right;">0</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The four serotypes of dengue virus (a mosquito-borne flavivirus) cause an acute febrile illness that occasionally results in hospitalization for treatment of hemorrhage (dengue fever; DF) or hemorrhage plus plasma leakage resulting in hypovolemia (dengue hemorrhagic fever; DHF). Hospitalization for these complications usually occurs during a second heterotypic infection, suggesting a role for dengue antibodies in pathogenesis. The major antigen of the virus, against which neutralizing, hemagglutination-inhibiting, and flavivirus cross-reactive antibodies are directed, is the envelope glycoprotein (E). Computer analysis revealed a 20 residue region of similarity in amino acid sequence between the dengue type 4 E and a family of clotting factors, including plasminogen, the prime mediator of fibrinolysis. Using synthetic peptides in ELISA, E antibodies that potentially bind plasminogen were previously detected in 75% of 40 Thai patients acutely infected with dengue virus type 1, 2, 3, or 4. Plasminogen cross-reactivity of dengue antibodies was shown to be specific for the related sites in E and plasminogen. The dengue E sequence with similarity to plasminogen is largely conserved within the currently known flavivirus E sequences. However, 15 Thai patients hospitalized for illness caused by Japanese encephalitis (JE) virus (a flavivirus not associated with hemorrhage) did not develop plasminogen cross-reactive antibodies, and this finding correlated with failure of JE antibodies to bind to the plasminogen cross-reactive site in E. An <i>in vitro</i> assay was developed to evaluate the possible effect of plasminogen cross-reactive antibodies in sera from dengue virus infected patients on plasmin activity in the presence or absence of α_2-antiplasmin, the naturally occurring plasmin antagonist. Preliminary results show that such antibodies have a minimal inhibitory effect on plasmin activity that is synergistically augmented in the presence of α_2-antiplasmin. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00571-02 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Amino Acid Substitution at the NS1-NS2A Cleavage Junction of Dengue Virus Polyprotein		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 33%;"> PI: Michèle Pethel </div> <div style="width: 33%;"> Microbiologist </div> <div style="width: 33%;"> LID, NIAID </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 33%;"> Others: Ching-Juh Lai, M.D. </div> <div style="width: 33%;"> Head, MVB Section </div> <div style="width: 33%;"> LID, NIAID </div> </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">1.0</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Deletion analysis of the NS1-NS2A region of the dengue virus polyprotein indicated that a minimum length of eight amino acids at the NS1 C-terminus is required for cleavage between non-structural proteins NS1 and NS2A to take place. Comparison of this eight amino acid sequence of dengue type 4 virus with the analogous sequences of other flaviviruses identified a sequence motif in which Ala(-1), Val(-3), Ser(-5), Val(-7), and Met/Leu(-8) are conserved. Amino acids at other positions vary. We were interested in determining whether the observed sequence motif is optimal for cleavage of the polyprotein at the NS1-NS2A junction. Mutants of NS1-NS2 were constructed that contained an amino acid substitution at different positions upstream of the cleavage site or at the immediate downstream position. The effect of these amino acid substitutions on cleavage of NS1-NS2A was then analyzed. The results showed that most substitutions of Ala(-1) or Val(-3) yielded predominantly uncleaved NS1-NS2A fusion protein. On the other hand, replacement of Thr(-2) had little or no effect on cleavage. These results are in agreement with the proposed consensus sequence deduced by comparison of sequences among flaviviruses. Several substitutions of Gly(+1) yielded reduced cleavage indicating that Gly at this position also plays a role in cleavage. Mutants that contain a single amino acid substitution in one of the remaining five positions have been constructed, and work is in progress to define their NS1-NS2 cleavage phenotype. A panel of amino acid substitution mutations that had a varying effect upon efficiency of NS1-NS2A cleavage were selected for incorporation into the full-length cDNA, and <i>in vitro</i> derived RNA transcripts were used to transfect cells and recover viable dengue virus mutants. These viable mutants exhibited growth restriction as revealed by plaque analysis. Evaluation of other viral growth properties of these mutants is in progress. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00572-02 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Dengue Virus Polyprotein NS3-NS4A-NS4B-NS5

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Annie Cahour, Ph.D. Visiting Associate LID, NIAID

Others: Barry Falgout, Ph.D. Senior Staff Fellow LID, NIAID
Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cleavage mechanism utilized for expression of the polyprotein NS3-NS4A-NS4B-NS5 domain of dengue virus was studied with the aim of elucidating the functional activities of these dengue virus proteins. For this purpose, recombinant vaccinia viruses v(NS2B-NS3-NS4A-NS4B-NS5), v(NS3-NS4A-NS4B-NS5), v(NS4A-NS4B-NS5), and v(NS4B-NS5) were constructed. These recombinants were used to infect cells and the labelled lysates were analyzed by NS3 or NS5 specific antiserum. Our findings indicated that NS2B is required for processing of the downstream nonstructural proteins. In the presence of NS2B supplied *in trans*, polyprotein NS3-NS4A-NS4B-NS5 was cleaved at the NS3-NS4A junction although less efficiently than at the NS4B-NS5 junction. The flavivirus NS4A-NS4B cleavage junction follows a long hydrophobic sequence. The polyprotein NS4A-NS4B-NS5 segment was properly cleaved at the NS4A-NS4B junction in the absence of other dengue viral functions such as the NS1-NS2A and NS2B-NS3 protease systems. However, v(NS3-NS4A-NS4B-NS5) expressed only the uncleaved polyprotein precursor. Thus, cleavage at the NS3-NS4A junction appears to be a prerequisite for cleavage at the downstream junction to take place. Finally, recombinants that expressed an uncleaved NS4B-NS5 polyprotein, such as NS3-NS4A-NS4B-NS5, NS4A-NS4B-NS5 or NS4B-NS5, produced properly cleaved NS5 when used for coinfection with v(NS2B-NS3 30%), or with v(NS2B) plus v(NS3). These results indicated that cleavage at the NS4B-NS5 junction of the polyprotein is mediated by NS2B and NS3 *in trans*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00597-01 LID</div>
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Construction and Characterization of Chimeric Dengue Viruses		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <div style="display: flex; justify-content: space-between; padding: 5px 0;"> <div>PI: Michael Bray, M.D.</div> <div>Senior Staff Fellow</div> <div>LID, NIAID</div> </div> <div style="display: flex; justify-content: space-between; padding: 5px 0; margin-top: 10px;"> <div>Others: Ching-Juh Lai, Ph.D.</div> <div>Head, MVB Section</div> <div>LID, NIAID</div> </div>		
COOPERATING UNITS <i>(if any)</i> 		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Molecular Viral Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">1.0</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">1.0</div>	OTHER: <div style="text-align: center; font-weight: bold;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p> Dengue virus, a flavivirus, causes human disease throughout tropical regions of the world. There are four serotypes; infection with one produces immunity only to that type. Serotype is a property of the viral structural proteins. The dengue viral genome, an 11 kilobase strand of positive-sense RNA, contains of three structural protein genes followed by a series of 7 non-structural protein genes. Earlier, we constructed a full-length cDNA copy of the entire dengue 4 genome, RNA transcripts from which were infectious when transfected into mammalian cells. We have now proceeded to replace the structural protein genes of this full-length clone with the structural protein genes of serotype 1 or 2 dengue virus, to create chimeric dengue 1/4 and dengue 2/4 cDNA genomes. We transfected cells with RNA transcribed from these templates, and recovered viruses which exhibited a hybrid phenotype. These viruses produced apparently authentic dengue 1 or dengue 2 structural proteins, respectively, together with dengue 4 non-structural proteins. The dengue 1/4 virus appeared to grow somewhat slower than wild-type type 4 virus, and produced smaller plaques on simian LLC-MK2 cells or mosquito cells. The dengue 2/4 chimera grew even more slowly than dengue type 4 virus, and produced smaller plaques. Evaluation of these viruses for mouse neurovirulence showed that wild-type dengue 4 and dengue 1 and chimera 1/4 lacked neurovirulence. However, the wild-type dengue 2 virus used in this experiment was mouse-brain adapted, and proved highly neurovirulent in suckling mice. The 2/4 chimeric virus, which expressed the structural proteins of the dengue 2 strain, was also neurovirulent, but its level of virulence was less than that of the wild-type type 2 virus. The attenuation properties of the 1/4 and 2/4 chimeras makes them interesting live virus vaccine candidates. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00598-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dengue Type 4/Type 2 (NS2A and/or NS2B) Chimeric Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Hiroshi Kawano, M.D. Visiting Associate LID, NIAID

Others: Ching-Juh Lai, Ph.D. Head, MVB Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The dengue virus genome codes for a polyprotein in the order of NH₂-C-PreM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH. Our previous studies indicated both that NS2A and NS2B play a role in the proteolytic processing of dengue virus nonstructural proteins. The homology of NS2A and NS2B among different dengue virus serotypes is low suggesting that an evolutionary divergence of dengue viruses has yielded a gene constellation that is optimal for replication of each serotype. We are interested in engineering stable dengue virus mutants that are restricted in viral replication because such mutants may exhibit reduced virulence and hence be useful for immunoprophylaxis. For these reasons we initiated construction of infectious dengue type 4 virus recombinant mutants that contain heterotypic dengue type 2 virus genes coding for NS2A, NS2B, or NS2A-NS2B. As a consequence of the mixed gene constellation of such chimeric viruses, it is possible that some of these recombinants might be restricted in virus replication *in vivo* and hence be attenuated. Dengue type 4 virus recombinant DNAs were constructed that coded for NS1(D2NS2A) NS2BNS3(30%), NS1NS2A(D2NS2B) NS3(30%), or NS1(D2NS2A-NS2B) NS3(30%) containing the indicated heterotypic dengue type 2 virus sequence. Functional analysis of these DNA constructs using the pTM-1/vTF7 transient expression system indicated that each of the three polyproteins containing the heterotypic dengue 2 sequence underwent apparently normal protein processing. Finally, full-length dengue type 4 cDNA constructs containing the heterotypic dengue type 2 sequence were prepared and used as templates for *in vitro* transcription. Preliminary results indicate that progeny chimeric dengue type 4 virus was recovered from cells transfected with the RNA transcripts that contained heterotypic dengue type 2 NS2A. Work is in progress to recover virus from other two chimeric, full-length cDNA constructs containing heterotypic dengue 2 sequence for NS2B or NS2A-NS2B.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00599-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cleavage of the Dengue Virus Capsid Protein by the Viral Protease, NS3

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lewis J. Markoff, M.D. Medical Officer LID, NIAID

Others: Barry N. Falgout, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The flaviviral protease NS3 is thought to cleave the flavivirus polyprotein after dibasic pairs of residues at the anchored precursor (anch C) virion capsid (virion C), NS2A-NS2B, NS2B-NS3, NS3-NS4A, and NS4B-NS5 sites. All of these cleavages have been demonstrated *in vivo* or *in vitro*, except that of virion C from anch C. To study the cleavage of anch C, we employed two plasmid vectors, pSTRUC, which contains dengue virus type 4 cDNA encoding C, prM, and the first 23 amino acids of E and pNS2B-NS3 which contains dengue virus type 4 cDNA encoding NS2B and the protease active domain of NS3. Both plasmid DNAs contain the T7 RNA polymerase promoter 5' to dengue cDNA sequences. RNA transcripts were prepared from both. Translation of pSTRUC transcripts in rabbit reticulocyte lysate in the presence of microsomal membranes results in cleavage at the C-prM site, mediated by signalase, producing anch C. The ectodomain of anch C was shown to be extraluminal with respect to ER. Translation of pNS2B/NS3 RNA transcripts in the presence of membranes results in augmented cleavage at the NS2B-NS3 site mediated by NS3 and in membrane association of cleaved NS2B and NS3. When pSTRUC and pNS2B/NS3 RNA transcripts are translated in the same reaction mix in the presence of membranes, the normally occurring cleavage events described above do occur, but cleavage of anch C to produce virion C, mediated by NS3 has not so far been observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT		Z01 AI 00600-01 LID
PERIOD COVERED		
October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)		
Engineering Viable Dengue Virus 3' Noncoding Region Deletion Mutants		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Ruhe Men, M.D.	Visiting Fellow LID, NIAID
Others:	Ching-Juh Lai, Ph.D.	Head, MVB Section LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH		
Laboratory of Infectious Diseases		
SECTION		
Molecular Viral Biology Section		
INSTITUTE AND LOCATION		
NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Sequence analysis indicated that dengue type 4 viral genome contains 384 nucleotides in the 3' non-coding region (NCR). The last 82 nucleotides are thought to assume a secondary structure probably required for initiation of RNA replication. Preceding this structure are sets of conserved sequences, designated CS-1 and CS-2, that are also found in other mosquito-borne flaviviruses. We have engineered a series of cDNA constructs containing deletions ranging from 29 to 201 nucleotides in length in the 3' NCR. Mutant RNA transcripts made from these DNA constructs were tested for infectivity by transfecting permissive tissue culture cells. The last 113 nucleotides including the secondary structure at the 3' end and the preceding CS-1 sequence apparently are essential for infectivity of dengue virus. In contrast, viable dengue virus was recovered from mutant DNA constructs containing a deletion in one or the other of the two CS-2 sequences. Many viable deletion mutants constructed in this manner were stable and produced plaques of reduced size on infected C6/35 mosquito cells compared to wild type virus. This suggests that these mutants are restricted for their replication. These mutants are now being characterized further for their replicative capacity and other properties in cultured cells as well as in infected animals. Dengue virus mutants that show reduced virulence for animals will be evaluated subsequently in humans for evidence of attenuation and immunogenicity.</p>		
12-33		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00333-09 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Longitudinal and Cross-Sectional Studies of Viral Gastroenteritis in Infants and Young Children		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID Others: Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID		
COOPERATING UNITS (if any) Children's Hospital National Medical Center, Washington, DC (Kim)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: right; margin-right: 50px;">0.7</div>	PROFESSIONAL: <div style="text-align: right; margin-right: 50px;"><.1</div>	OTHER: <div style="text-align: right; margin-right: 50px;">0.7</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Rotaviruses have been studied extensively in many parts of the world predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness in infants and young children. There has been a paucity of longitudinal viral gastroenteritis studies that yield not only important "denominator" data but also valuable insights into the natural history of a pathogen or illness, with special emphasis on epidemiologic, immunologic and laboratory information. We, therefore, initiated an intensive examination of anal swab and serum specimens obtained during a previous LID long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for homeless, but otherwise normal children. Anal swabs and blood specimens were obtained routinely from the youngest age groups, which ranged from 6 months to about 5 years of age during various periods of the study. Surveillance was carried out by a trained medical staff. Children were housed in cottages according to their age. Rectal temperatures were obtained on each child daily; routinely, a physician examined any child with a rectal temperature of 100.6°F or greater. Careful medical records were kept by the nursing staff on all children. Thus, with this background, we plan to investigate the natural history of rotavirus infections in a longitudinal manner employing newly developed techniques such as serotyping of rotaviruses with VP7 specific monoclonal antibodies and determining the epitope-specific serologic response in sequential sera to determine the scope of homotypic and heterotypic responses. In addition with the availability of rotavirus strains obtained over 20 years ago, it is planned to compare such strains with current isolates at the genetic level.</p> <p>In addition, there has been increased emphasis placed on the role of enteric adenoviruses and astroviruses as etiologic agents of gastroenteritis of infants and young children. With the ready availability of assays for detection of both of these groups of agents, we are planning to expand our longitudinal studies to evaluate the role of these viruses in the etiology of pediatric gastroenteritis. Also, the role of these agents will be examined in a cross-sectional manner by examining specimens from infants and young children hospitalized with diarrheal illness at the Children's Hospital National Medical Center, Washington, DC.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00339-10 LID</div>												
PERIOD COVERED October 1, 1990 to September 30, 1991														
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Isolation and Serotypic Characterization of Human and Animal Rotaviruses														
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Yasutaka Hoshino, D.V.M.</td> <td style="width: 30%;">Visiting Scientist</td> <td style="width: 20%;">LID, NIAID</td> </tr> <tr> <td>Others:</td> <td>Mitzi Sereno</td> <td>Microbiologist</td> <td>LID, NIAID</td> </tr> <tr> <td></td> <td>Albert Z. Kapikian, M.D.</td> <td>Head, Epid. Section</td> <td>LID, NIAID</td> </tr> </table>			PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID	Others:	Mitzi Sereno	Microbiologist	LID, NIAID		Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID
PI:	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID											
Others:	Mitzi Sereno	Microbiologist	LID, NIAID											
	Albert Z. Kapikian, M.D.	Head, Epid. Section	LID, NIAID											
COOPERATING UNITS <i>(if any)</i> Ohio Agricultural Research and Development Center, Ohio State University (Saif)														
LAB/BRANCH Laboratory of Infectious Diseases														
SECTION Epidemiology Section														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892														
TOTAL MAN-YEARS: <div style="text-align: center;">1.1</div>	PROFESSIONAL: <div style="text-align: center;">0.3</div>	OTHER: <div style="text-align: center;">0.8</div>												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input checked="" type="checkbox"/> (b) Human tissues</td> <td><input type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
<input type="checkbox"/> (a) Human subjects	<input checked="" type="checkbox"/> (b) Human tissues	<input type="checkbox"/> (c) Neither												
<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>Rotavirus can now be grown readily in cell cultures by techniques originally described by Japanese investigators which include pre-treatment of virus with trypsin, incorporation of trypsin in the maintenance medium, and incubation of roller tube cultures on a roller apparatus. Our objectives in this project are three-fold: (1) to cultivate directly in cell cultures a variety of human and animal rotavirus strains derived from diverse geographical areas and populations; (2) to define serotypic diversity and similarity among these viruses based on their VP4 and VP7 specificities; and (3) to select and develop potential rotavirus vaccine candidates (including direct cell-culture isolates or laboratory produced reassortant strains). In this project, the serotype of rotavirus isolates was characterized by plaque-reduction neutralization (PRN) assay.</p> <p>Three single VP7 gene substitution reassortants were generated using a porcine rotavirus Gottfried strain (VP7 serotype 4) and human rotavirus strains D (VP7 serotype 1), DS-1 (VP7 serotype 2), or M (VP7 serotype 3), each of which possesses only the VP7 gene of D, DS-1, or M with the remaining ten genes being derived from the Gottfried strain. Hyperimmune guinea pig antiserum raised against the reassortant strain Gottfried x DS-1, neutralized not only the parental strains Gottfried and DS-1 but also human rotaviruses belonging to VP7 serotype 1 (Wa strain), 3 (P strain), or 4 (VA70 and ST3 strains).</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00340-10 LID</div>
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Studies of Rotavirus Pathogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 40%;"> PI: Yasutaka Hoshino, D.V.M. </div> <div style="width: 30%;"> Visiting Scientist </div> <div style="width: 30%;"> LID, NIAID </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 40%;"> Others: Mitzi M. Sereno Albert Z. Kapikian, M.D. </div> <div style="width: 30%;"> Microbiologist Head, Epid. Section </div> <div style="width: 30%;"> LID, NIAID LID, NIAID </div> </div>		
COOPERATING UNITS (if any) Ohio Agricultural and Development Center, Ohio State University, Wooster, OH (Saif)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">0.8</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">0.3</div>	OTHER: <div style="text-align: center; font-weight: bold;">0.5</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Continued efforts have been made to delineate the genetic mechanisms underlying virulence of rotavirus in the newborn gnotobiotic piglet model. In addition to the three genes encoding rotavirus outer capsid protein VP4 or VP7 or nonstructural protein NS28, a fourth gene (gene number three) encoding a core protein appears to play an important role in determining the virulence of rotavirus in the porcine model. Information obtained from this study may have important implications for the development of effective and safe rotavirus vaccine strategies.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00341-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Live Attenuated Rotavirus Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

Others: Jorge Flores, M.D. Visiting Scientist LID, NIAID
Kim Y. Green, Ph.D. Senior Staff Fellow LID, NIAID
Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID
Mario Gorziglia, Ph.D. Visiting Scientist LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

Instituto de Biomedicina, Caracas, Venezuela (Pérez-Schael); Univ. of Rochester (Dolin, Madore, Christy); Johns Hopkins Univ. (Midthun, Clements, Halsey, Black); Univ. of Tampere (Vesikari, Ruuska), Vanderbilt Univ. (Wright); Wyeth-Ayerst Laboratories (Davidson); Secretic Laboratories

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

0.7

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The "Jennerian" approach to vaccination, which involves the use of a live virus vaccine strain derived from a non-human host, has been evaluated in clinical trials using surrogate rotavirus strains of bovine origin (by others) or of simian [rhesus monkey] origin (by the Epidemiology Section, LID). This strategy has had limited success because serotype-specific immunity against each of the four epidemiologically important human rotavirus VP7 serotypes could not be achieved consistently in infants less than 6 months of age who had not undergone prior rotavirus infection. Protection was observed when the rhesus rotavirus (RRV) vaccine (VP7 serotype 3) was used to immunize young infants who later were naturally exposed to human rotavirus strains of the same VP7 serotype. However, in other trials in which rotavirus with a VP7 serotype 1 predominated in the community, vaccine efficacy was variable. Thus, most recent clinical trials have employed a "modified Jennerian" approach in which individual reassortant strains initially and later a quadrivalent vaccine of broader antigenic coverage (that includes viruses of VP7 serotype 1, 2, 3, and 4) was used for immunization. This vaccine contains RRV (serotype 3) and reassortant rotaviruses containing 10 RRV genes and a single human rotavirus gene that encodes VP7 serotype 1, 2, or 4 specificity. Encouraging but variable results have been observed in efficacy trials with individual reassortant vaccines. In collaborative studies, in a field trial in Finland where VP7 serotype 1 strains were predominant, the protective efficacy of a reassortant vaccine with VP7 serotype 1 specificity was moderately high in the first but not the second rotavirus "season," whereas that of a reassortant vaccine with VP7 serotype 2 specificity was moderately high for both "seasons." Vaccine efficacy was observed only in individuals who developed a seroresponse after vaccination. The heterotypic protection was unexpected and is under investigation. Further successes as well as failures of this approach were observed in other locations. Analysis of the efficacy of the quadrivalent vaccine are in progress. In addition, a naturally attenuated human rotavirus strain, M37, with a VP7 specificity of serotype 1 and a unique VP4 specificity shared by other neonatal strains belonging to VP7 serotype 1, 2, 3, or 4, is also under evaluation. Phase I trials of the human rotavirus M37 live vaccine have been completed successfully. In a preliminary phase II trial in Finland this vaccine failed to induce protection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00342-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

Epidemiology Division, US Naval Medical Research Institute, Bethesda, MD, and Cairo, Egypt

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The electron microscope is an integral component of a laboratory engaged in the study of gastroenteritis viruses. Two major groups of gastroenteritis viruses--the 27nm Norwalk virus and the 70nm human rotaviruses--were discovered at NIH and in Australia, respectively, (as well as the hepatitis A virus at NIH, in collaboration with the Hepatitis Section) by the use of electron microscope techniques. Indeed, in this era of tissue culture virology, these agents were discovered without the use of an *in vitro* tissue culture system, because they could not be grown from clinical specimens in cell culture. The term, "direct virology," is apt to describe this method of examining viruses from clinical specimens by electron microscopy.

Although second and third generation tests have been developed for the detection of the Norwalk group of viruses and the rotaviruses, the electron microscope is still an indispensable tool for the study of these gastroenteritis viruses. It is also the most rapid diagnostic method for detection of rotavirus from a clinical specimen and is the only method available for detecting infection with certain 27nm or similar small round virus-like particles from individuals with viruses associated with epidemic nonbacterial gastroenteritis. In addition, it is the only method that is capable of detecting all known gastroenteritis viruses (e.g., group A or non-group A rotavirus, Norwalk-like viruses, adenoviruses, astroviruses, caliciviruses) by examination of a single stool specimen. This became especially apparent when stools from Desert Storm troops who developed acute gastroenteritis needed to be evaluated for the presence of viral agents. It also is important for: (i) providing direct visualization of virus particles from density gradients (to establish their morphologic appearance, e.g., single or double capsid, integrity of capsid structure, and to determine presence or absence of particles or their quantitation); (ii) providing direct visualization of particles from unusual clinical specimens to determine their identity, if feasible; (iii) attempting to visualize the site of activity of antibodies such as monoclonal antibodies or recombinant virus induced antibodies; and (iv) serologic studies performed by immune electron microscopy to determine the antigenic relationships of fastidious gastroenteritis agents that cannot be propagated in cell culture such as the human group C rotaviruses, and the Norwalk group of agents. However, its most important and creative role is in its application to the detection of new, heretofore unknown, agents of acute infectious gastroenteritis and other diseases as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00343-10 LID</div>
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> The Role of Norwalk Virus and Related Norwalk-Like Viruses in Viral Gastroenteritis		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <div style="display: flex; justify-content: space-between; padding: 5px 0;"> <div>PI: Kim Green, Ph.D.</div> <div>Sr. Staff Fellow</div> <div>LID, NIAID</div> </div> <div style="display: flex; justify-content: space-between; padding: 5px 0; margin-top: 10px;"> <div>Others: Albert Z. Kapikian, M.D.</div> <div>Head, Epid. Section</div> <div>LID, NIAID</div> </div>		
COOPERATING UNITS <i>(if any)</i>		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">0.6</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;"><.1</div>	OTHER: <div style="text-align: center; font-weight: bold;">0.6</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <div style="padding: 10px;"> <p>Norwalk virus is one of a group of small, noncultivable, 27nm viruses that are important etiologic agents of epidemic gastroenteritis in older children and adults. An outbreak of epidemic diarrhea in military personnel was analyzed in our laboratory and the etiologic agent was identified as a "Norwalk-like" agent. Further analysis of this etiologic agent is in progress.</p> </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00346-10 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Genetic Characterization of the Rotavirus VP4 Gene by Sequencing and Hybridization Techniques		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI:	Jorge Flores, M.D.	Visiting Scientist LID, NIAID
Others:	A. Duncan Steele, Ph.D.	Visiting Fellow LID, NIAID
COOPERATING UNITS <i>(if any)</i> <div style="height: 40px;"></div>		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>It has been suggested that the asymptomatic nature of most rotavirus infections that occur in newborn nurseries is a result of the low virulence of strains that are readily transmitted and persist in the nursery without calling attention to their presence or without requiring intervention to interrupt transmission. The high degree of sequence relatedness between the fourth gene of the M37 strain (derived from an asymptomatic newborn in Venezuela) and other strains recovered from asymptomatic neonatal infections in other locations suggested that this gene was related to the putative low virulence of the neonatal strains. Furthermore, the gene 4 of neonatal strains differs significantly in sequence from that of strains with similar VP7 serotype specificity isolated from older infants with diarrhea. To extend these observations, we obtained additional nursery specimens from various locations as well as strains recovered from older infants with diarrhea and examined them by hybridization and sequencing techniques. The results indicate that: (i) the M37 gene 4 allele is associated with long-term persistent rotavirus transmission in newborn nurseries that is not associated with significant diarrheal disease, (ii) other strains sharing their gene 4 with wild type rotaviruses associated with diarrhea in older infants may be found in asymptomatic neonates, but these strains have not been associated with persistence and silent transmission of rotavirus in newborn nurseries, and (iii) strains with a M37-like gene 4 may cause diarrhea in older infants, but this is an infrequent event.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00451-07 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological and Molecular Characterization of Rotavirus Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid Section LID, NIAID

Others:	Mario Gorziglia, Ph.D.	Visiting Associate	LID, NIAID
	Koki Taniguchi, Ph.D.	Visiting Associate	LID, NIAID
	Kazuo Nishikawa, M.D.	Visiting Associate	LID, NIAID
	Kim Green, Ph.D.	Staff Fellow	LID, NIAID
	Yasutaka Hoshino, D.V.M.	Visiting Scientist	LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00478-06 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Rotavirus/Adenovirus Recombinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jorge Flores, M.D. Visiting Scientist LID, NIAID

Others: Gisela Larralde Guest Researcher LID, NIAID
Mario Gorziglia, Ph.D. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1990-91

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00507-05 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Characterization of Rotavirus Serotypes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Kim Y. Green, Ph.D	Sr. Staff Fellow LID, NIAID
Others:	Yuan Qian, M.D.	Visiting Associate LID, NIAID
COOPERATING UNITS (if any) University of Rochester, Rochester, NY (Madore & Dolin); University of Tampere, Tampere, Finland (Vesikari & Ruuska)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.0	PROFESSIONAL: 0.3 OTHER: 0.7
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Rotavirus outer capsid proteins VP7 and VP4 independently induce the development of protective neutralizing antibodies in the host. Hence, the molecular basis for antigenic differences among VP7s and VP4s is relevant to rotavirus vaccine development. Circulating rotavirus strains from rotavirus vaccine trials were examined by sequence analysis to determine whether antigenic variation plays a role in variable vaccine efficacy that has been observed in several clinical trials. Strains from three different rotavirus vaccine efficacy studies were examined this year: a RRV (serotype 3 VP7) trial in Rochester, New York where protection was observed against a circulating serotype 1 strain; a RRV (serotype 3 VP7) trial in Rochester where protection was not observed against a circulating serotype 1 strain; and an M37 (serotype 1 VP7) study in Finland where protection was not observed against a circulating serotype 1 strain. Comparison of the VP7 sequences of the strains derived from "vaccine failures" or placebo recipients in these studies with those of the vaccine strain suggest that antigenic variation may play a role in the ability of a circulating strain to escape vaccine-induced immunity. However, additional sequence analysis in concert with examination of the serologic responses of vaccinees will be required to confirm this association.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00532-04 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Rotavirus Outer Capsid Protein VP4 by a Baculovirus Recombinant

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mario Gorziglia, Ph.D. Visiting Scientist LID, NIAID

Others: Gisela Larralde Guest Researcher LID, NIAID
Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00533-04 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Rotavirus Proteins with Monoclonal Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kim Green, Ph.D. Senior Staff Fellow LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epidemiology Section LID, NIAID

COOPERATING UNITS (if any)

University of Tampere, Tampere, Finland (Vesikari & Ruuska)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Monoclonal antibodies have proved to be important reagents for the study of rotavirus antigens. Several monoclonal antibodies have been adapted in our laboratory for use in an epitope-blocking immunoassay to analyze antigenic site-specific antibody responses. This assay has been applied to the analysis of sera from various sources including rotavirus vaccine trials and human volunteer and animal studies in an attempt to better understand the development of immunity in the host and identify correlates of resistance to rotavirus illness. Monoclonal antibodies are also vital in identification of serotypes of rotavirus strains circulating in the community during rotavirus vaccine trials. These studies are providing critical information on the epidemiology of rotavirus serotypes and the role of serotype-specific immunity induced by rotavirus vaccine candidates.

Analysis of sera of infant vaccinees who developed rotavirus illness during post-immunization surveillance (i.e., "vaccine failures") indicated that failure of vaccinees to develop neutralizing neutralization epitope blocking antibodies to the illness strain was a major correlate of susceptibility to the illness producing rotavirus.

Further study of adult volunteers who were challenged with a virulent human serotype 1 rotavirus indicated that resistance to rotavirus infection and diarrhea was strongly associated with antibodies to a serotype 1 or serotype 3 neutralization epitope that maps to amino acid 94 of the outer capsid protein VP7.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00558-03 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prediction of Rotavirus Serotypes by Hybridization to Specific Probes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jorge Flores, M.D. Visiting Scientist LID, NIAID

Others: A. Duncan Steele Visiting Fellow LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A surrogate method to identify the serotype specificity of rotaviruses was developed. The assay is based on hybridization of PCR generated DNA probes corresponding to hyperdivergent regions of the VP7 and VP4 rotavirus genes to RNAs extracted from test specimens and immobilized on nylon membranes. To generate the probes, specific primers are employed to selectively amplify regions of the VP4 or VP7 gene which are associated with neutralization specificity. The inclusion of P³²-deoxy ATP in the PCR yields probes of high specific activity. We have developed probes corresponding to most of the 12 known VP7 serotypes and the five known gene 4 alleles and have employed these probes in an analysis of various collections of rotavirus field specimens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 AI 00573-02 LID</div>
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression of Rotavirus Proteins in <i>Salmonella</i> Bacteria		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Kim Green, Ph.D.	Sr. Staff Fellow LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist LID, NIAID
COOPERATING UNITS (if any) University of Maryland, Baltimore, Maryland (Levine & Hone)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goal of this project is to assess the feasibility of a recombinant rotavirus vaccine using attenuated <i>Salmonella</i> bacteria as an antigen delivery system. In the pursuit of this goal, antigens involved in the development of broadly-reactive immunity are being studied. The genes encoding VP4 (an outer capsid protein containing neutralization antigens) and VP6 (an inner capsid protein containing group antigens) of rotavirus strain KU have been expressed in <i>E. coli</i> and <i>Salmonella typhimurium</i> . A mouse animal model is being developed to analyze these constructs as immunogens and protective antigens.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00574-02 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression and Distribution of Conserved and Serotype-Specific Epitopes on Rotavirus VP8		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Gisela Larralde	Guest Researcher LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Three cDNA clones of approximately 300 nucleotides each and comprising the VP8 region of the VP4 protein of human rotavirus (HRV) strain KU, were constructed, inserted into the pGEMEX-1 plasmid and expressed in <i>Escherichia coli</i>. Immunoblot analysis of the corresponding peptides designated A (amino acid 1 to 105), B (amino acid 85 to 183), or C (amino acid 153 to 254), were performed using antisera to KU or Wa (VP4 serotype 1A), DS-1 (VP4 serotype 1B), or 1076 or M37 (VP4 serotype 2) rotavirus strains. These hyperimmune sera were prepared in guinea pigs inoculated with purified virus or expressed VP4 protein. The immunoblot analysis showed that epitopes located in fragment B were VP4 serotype and subtype specific and suggested that this fragment may be responsible for VP4 serotype and subtype specificity. In contrast, fragments A and C contained epitopes that were conserved among different human rotavirus VP4 serotypes. The high level of cross-reactivity observed in neutralization assay with antisera to the expressed VP8 protein may result from antibody to the conserved A and C fragments.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00575-02 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of the Outer Capsid Protein of Human Rotaviruses in Infectivity		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Gisela Larralde, M.S.	Guest Researcher LID, NIAID
Others:	Mario Gorziglia, Ph.D.	Visiting Scientist LID, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither		
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
INACTIVE 1990-91		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00576-02 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Serotype Analysis and Characterization of Rotaviruses from Malaysia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Nassar Rasool, Ph.D. Guest Researcher LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID
Mario Gorziglia, Ph.D. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

<0.1

PROFESSIONAL:

<0.1

OTHER:

<0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A total of 341 stool specimens known to be positive for group A rotaviruses by RNA electrophoresis, obtained from various locations in Malaysia from hospitalized children below the age of five years who had gastroenteritis, was brought to the NIH for determination of the serotypes circulating in Malaysia and for further characterization by molecular biologic techniques. Most of the specimens (87%) were collected in the 1987/88 season; the remainder (13%) were collected in 1977, 78, 83, 84, and 86. Serotype analysis was performed on 306 specimens; 35 specimens were antigen-negative when tested by a pre-post confirmatory ELISA and were, thus, not analyzed. One hundred eighty-two of the 306 specimens (60%) could be serotyped: 15% were serotype 1, 4% serotype 2, 4% serotype 3, 71% serotype 4, and 6% exhibited multiple serotype-specificities. Most of the typable specimens (93%) were from the 1987/88 season. The remaining 7% came from 1978, 1984, and 1986 (none were typed from 1977 and 1983). Serotype 4, followed by serotype 1 rotavirus, predominated in central and north-western Peninsular Malaysia in the 1987/88 season, while it appeared from a limited number of strains that a more even serotype distribution was observed in East Malaysia. Eighty-two percent of stool specimens that were serotyped could be adapted to grow in primary African Green monkey kidney (AGMK) cells after one or two passages, whereas only 44% of the specimens that did not serotype were adapted successfully. It was noteworthy that 32% of rotavirus ELISA-negative stools were adapted to growth in AGMK cells and found to be rotavirus positive by ELISA. Of 190 specimens that grew after second passage in AGMK cells, 78% could be adapted to growth in MA104 cells after one passage; 10% of these were specimens that did not serotype. The latter, plus two possible serotype 1 monotypes and specimens that reacted with more than one serotype-specific monoclonal antibody are potential candidates for molecular characterization of their VP7 genes. Some of these are being amplified in MA104 roller cultures. In addition, all rotavirus ELISA-positive MA104 passages will be subjected to VP4 typing to determine the distribution of VP4 serotypes in Malaysia and to detect potential candidates for virus amplification and VP4 gene sequencing.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00577-02 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Characterization of the VP4 Gene of Strain 69M, a New Human Rotavirus Serotype

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yuan Qian, M.D. Visiting Associate LID, NIAID

Others: Kim Green, Ph.D. Senior Staff Fellow LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00582-02 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression and Recovery of Recombinant Rotavirus VP4 from Insect Cell Cultures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mario Gorziglia, Ph.D. Visiting Associate LID, NIAID

Others: Luis G. Juarbe-Osorio Guest Researcher LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The possibility of producing and purifying large quantities of recombinant rotavirus VP4 from insect cell cultures was investigated. *Spodoptera frugiperda* (Sf9) cells were grown in large spinner flasks and infected with a baculovirus expressing the rotavirus OSU VP4 gene. The expressed VP4 subsequently was purified from such cultures to determine the yield of recombinant protein. Conditions for growth of cells and attainment of a high yield of VP4 were optimized to improve the expression and recovery of VP4. The fate of the expressed VP4 antigen and its degradation were examined. Also, a practical purification scheme was developed to maximize the yield of VP4 from insect cell cultures and to reduce cost and complexity of a potential industrial-scale purification process.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00583-02 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of the Outer Capsid Protein VP4 of Human and Porcine Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Baoguang Li, Ph.D. Visiting Associate LID, NIAID

Others: Mario Gorziglia, Ph.D. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human rotavirus strain K8 not only possesses a unique VP4 with only 60-70% amino acid sequence homology with other human rotavirus strains but also appears to represent a distinct VP4 serotype in one-way neutralization assays. In previous studies, strain K8 was provisionally classified as VP4 serotype 3, but this remained to be confirmed in reciprocal neutralization tests. To achieve this goal, the cDNA of VP8 (the VP4 serotype-specific subunit) was inserted into the pGEMEX plasmid vector (Promega) and expressed in *E. coli* (JM109). Neutralization tests with antiserum to the *E. coli*-expressed K8 VP8 supported the classification of K8 as a distinct serotype. In addition, the cDNAs that represent K8 VP4 or its subunit VP8, or Gottfried VP4 were inserted into a baculovirus expression vector. The cloned gene was expressed at high yield in *Spodoptera frugiperda* cells. The guinea pigs immunized with baculovirus expressed VP4 of the K8 strain developed a high level of neutralizing antibodies to the K8 strain. Heterotypic neutralization assays with other human strains are currently underway. Furthermore, the VP8 of human rotavirus strain K8 and the VP4 of porcine rotavirus Gottfried were also expressed in a baculovirus expression vector. Immunization of animals with the expressed proteins is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00601-01 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Recombinant BCG-VP8 Vaccines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Mario Gorziglia, Ph.D.	Visiting Scientist LID, NIAID
Others:	Gisela Larralde	Guest Researcher LID, NIAID
COOPERATING UNITS (if any) Medimmune Laboratory (Stover)		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Epidemiology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Rotaviruses are the major cause of severe diarrhea in infants and young children in both developed and developing countries. Two outer capsid rotavirus proteins, VP7 and VP4, are associated with the induction of neutralizing antibodies which have been shown in experimental animals to be associated with resistance to illness. Serotype specificity is associated primarily with VP7. However, recent studies have elucidated the antigenic relationships of the VP4 of human rotaviruses which appear to be less polymorphic than VP7. Human rotavirus strains that are associated with symptomatic infection and that exhibit VP7 specificity of serotype 1, 2, 3, 4 or 9 each possess a similar VP4 as determined by neutralization assay. Objectives of this study were: (i) to use the VP8 subunit of VP4 to develop a BCG-VP8 recombinant and evaluate its ability to induce specific rotavirus neutralizing antibodies in experimental animals, and (ii) to utilize the information thus obtained for the development of a human rotavirus vaccine for oral administration.</p> <p>The cDNA representing the VP8 subunit of outer capsid rotavirus protein VP4 from the human rotavirus KU strain was cloned in three different BCG vectors. The VP8 subunit expressed in these systems was recognized in an immunoblot assay by antibodies present in hyperimmune antiserum to: (i) human rotavirus strain Wa, (ii) expressed KU-VP4, and (iii) expressed KU-VP8, a cleavage subunit of VP4. These observations suggest that the VP8 subunit was expressed in an antigenically correct form by the recombinant as authentic VP8.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00602-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Rotavirus Outer Capsid Protein VP4 by Recombinant-Adenovirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mario Gorziglia, Ph.D. Visiting Scientist LID, NIAID

Others: Gisela Larralde Guest Researcher LID, NIAID
Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

Wyeth-Ayerst Research Laboratories (Davis & Selling)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.3

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The outer capsid protein VP4 of porcine rotavirus OSU was chosen as a model to study the immunogenicity of this protein expressed by an adenovirus vector. The ability of adenovirus to be administered orally makes the potential use of adenovirus vectors for vaccine production particularly attractive. In addition, live adenovirus vaccines have already been shown to be safe in humans when administered orally in enteric coated capsules.

The full-length cDNA of the VP4 of the porcine rotavirus OSU strain was cloned into adenovirus type 5 at a location downstream of the E3 promoter. In this viable recombinant the rotavirus VP4 sequence was substituted for sequences in the adenovirus E3 region. The plaque-purified recombinant (Ad5-OSU VP4), expressed an antigenically authentic VP4 protein as determined by immunoprecipitation. The 84 kDa protein had the same electrophoretic mobility as the native VP4 of OSU rotavirus grown in MA104 cells. Preliminary results from a study in which cotton rats were infected intranasally with live Ad5-OSU VP4 indicated that the animals developed a low level of neutralizing antibodies to the OSU porcine rotavirus.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00603-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibodies Directed to VP8 Subunit of VP4

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Mario Gorziglia, Ph.D. Visiting Scientist LID, NIAID

Others: Ronald Jones Biologist LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously, antisera against recombinant outer capsid rotavirus VP4 proteins were used to study the VP4 antigenic polymorphism among human rotavirus strains. Three distinct serotypes and one subtype of the VP4 protein were identified among 17 human rotavirus strains that contained representatives of five different VP7 serotypes.

Immunization of mice with the VP8 cleavage subunit of VP4 of the human rotavirus KU, DS-1, 1076, or K8 strain induced antibodies that neutralized the rotavirus from which the VP4 subunit was derived. In one fusion with the spleen of a mouse inoculated with VP8-DS-1, 14 hybridomas were detected that reacted with rotavirus by ELISA. Some mAbs only recognized the homologous human rotavirus DS-1 strain, whereas others were cross-reactive. These results suggest that the VP8 subunit of VP4 contains specific regions which can be useful in distinguishing VP4 serotypes among human rotaviruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00604-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cold-Adaptation of Human Rotaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID

Others: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

COOPERATING UNITS (if any)

National Institutes of Health, Tokyo, Japan (Matsuno)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three human rotavirus cell-culture isolates (strains D [VP7:1, VP4:1A], DS-1 [VP7:2, VP4:1B], and 202 [VP7:4, VP4:1B]) as well as two human x human rotavirus reassortants (Wa x P [VP7:3, VP4:1A] and Wa x DS-1 [VP7:2, VP4:1A]) have been successfully adapted to grow in primary African Green monkey kidney cells at the suboptimal temperature of 30°C, 28°C, or 26°C. These rotavirus strains were cold-adapted in order to determine whether cold-adapted (*ca*) mutants selected by this strategy are attenuated and suitable for use in a live virus vaccine. The D and the Wa x P strains were passaged 10 times either at 28°C or 26°C and are being plaque-purified. Analysis of the efficiency of plaque formation at various temperatures established that the 26°C cold-adapted D strain is indeed a *ca* mutant; it produced plaques at 26°C, whereas its parent did not. In addition, this mutant is also temperature sensitive having a shut-off temperature for plaque formation of 37°C. The DS-1 strain (passaged 12 times at 28°C) and Wa x DS-1 reassortant (passaged 10 times at 28°C) are also being plaque-purified. Selected human rotaviruses including the D, Wa x P, and Wa x DS-1 have successfully been adapted to growth in DBS-FR_hL-2 cells, which is a cell substrate suitable for preparation of vaccines for use in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00605-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Infection of Chimpanzees by Human Rotavirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Albert Z. Kapikian, M.D. Head, Epid. Section LID, NIAID

Others: Yasutaka Hoshino, D.V.M. Visiting Scientist LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

Georgetown University/Twinbrook (London); SEMA Laboratories, Rockville, MD (Bradbury)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

<0.1

PROFESSIONAL:

<0.1

OTHER:

<0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses are consistently shown to be the single most important etiologic agents of severe diarrhea of infants and young children world-wide. We are engaged in studies to develop a human rotavirus vaccine with the goal of preventing or modifying the severe toll resulting from rotavirus illness; it is estimated that 873,000 infants and young children under five years of age die from rotavirus diarrhea annually in the developing countries of the world (Institute of Medicine Report). Our current vaccination strategy is comprised of the use of an animal rotavirus strain (derived from a rhesus monkey) in combination with three human rotavirus-rhesus rotavirus reassortant strains to form a quadrivalent vaccine in an attempt to achieve protection against each of the four epidemiologically important rotavirus serotypes. This "Jennerian" and "modified Jennerian" approach to vaccination has met with variable success in field trials and is continuing to be evaluated. We are now considering the use of a cold-adapted human rotavirus strain(s) as a candidate vaccine because it may yield a higher degree of protection than the aforementioned vaccine, because the cold-adapted strain(s) possess the human rotavirus VP4 (whereas the quadrivalent vaccine possesses the rhesus rotavirus VP4), an important outer capsid protein that may be important in the induction of heterotypic immunity. VP4 is one of two outer capsid rotavirus proteins associated with protection against rotavirus disease in animal models. In order to determine if the cold-adapted human rotavirus strain is attenuated, an important first step is the development of a suitable animal model is needed that can be reproducibly infected and can develop diarrhea following administration of a virulent human rotavirus strain by the alimentary route. Therefore, we administered a serotype 1 human rotavirus strain (that was known to be virulent in prior studies in adult human volunteers) to two chimpanzees by the alimentary route via gastric tube; one of them: (1) developed loose stools on the fourth, fifth, and sixth days after inoculation; (2) shed rotavirus on day 2 to at least day 7; and (3) developed a seroresponse to the serotype 1 virus by plaque reduction neutralization (PRN) assay. The other chimpanzee developed loose stools beginning on the third or fourth day and subsiding before the fifth day after inoculation. The latter animal did not shed rotavirus and failed to develop a seroresponse to serotype 1 rotavirus by PRN assay.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00606-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Neonatal Rotavirus Strains Detected in South Africa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Duncan Steele, Ph.D. Visiting Fellow LID, NIAID

Others: Jorge Flores, M.D. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.1

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent reports of the occurrence of sporadic symptomatic infections in neonates with rotavirus strains bearing the M37-like VP4 gene have stimulated interest regarding the putative attenuated nature of neonatal rotaviruses. A number of these strains are at present being adapted to tissue culture. The nucleic acid sequence analysis of these strains associated with symptomatic infection in neonates may provide data which would help to explain the obscured attenuated nature of the majority of rotavirus strains recovered from neonates who are infected in newborn nurseries.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00323-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Expression of Parainfluenza Type 3 Virus Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph.D. Senior Staff Fellow LID, NIAID

Others: Genevieve Mottet Special Volunteer LID, NIAID

COOPERATING UNITS (if any)

Upjohn Laboratories, Kalamazoo, MI (Nicholas)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.75

PROFESSIONAL:

0.25

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments were initiated to characterize cell-mediated immune responses to human parainfluenza virus type 3 (PIV3). BALB/c mice were infected intranasally with RSV and splenocytes were isolated 3 to 6 weeks later. Upon stimulation *in vitro* with PIV3, cytolytic activity was demonstrated which was PIV3-specific, MHC class I-restricted and CD8 positive. The antigen specificities of these cytotoxic T cells (CTL) were investigated using recombinant vaccinia viruses which express the PIV3 hemagglutinin-neuraminidase (HN) glycoprotein, fusion (F) glycoprotein or major nucleocapsid (NP) protein (vac-HN, vac-F, or vac-NP). In studies to date, vac-HN and vac-F were tested for the ability to induce CTL upon intraperitoneal immunization, or for the ability to prepare targets *in vitro* for lysis by CTL from PIV3-infected mice. Neither vac-HN nor vac-F were effective in inducing PIV3-specific CTL, and neither recombinant was effective in preparing targets *in vitro*. Vac-NP remains to be tested. These results suggest that the HN and F glycoproteins, which are the major neutralization and protective antigens, are not significant CTL antigens in the BALB/c mouse. Presumably, one or more other viral proteins are responsible for inducing PIV3-specific CTLs, as will be elucidated in further studies using additional PIV3-vaccinia virus recombinants. This will form the basis for further studies to evaluate the relative abilities of CTL versus neutralizing antibodies to restrict PIV3 infection in the mouse model.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00324-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory Studies of Influenza Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: Robert M. Chanock, M.D. Chief LID, NIAID
 Kanta Subbarao, M.D. Senior Staff Fellow LID, NIAID
 Cassandra Lawson, Ph.D. Visiting Associate LID, NIAID

COOPERATING UNITS (if any)

University of Rochester (Treanor); Mt. Sinai School of Medicine (Palese); St. Jude Children's Hospital (Webster)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.7

PROFESSIONAL:

1.95

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Stability of mutant genes of the cold-adapted influenza virus donor strain. The genetic stability of the PB2 gene of the cold-adapted (*ca*) donor virus, used to prepare reassortant vaccine strains, was studied by subjecting a single gene (PB2) reassortant virus to serial passage in cell culture at a non-permissive temperature. Genetic and sequence analysis of *ts*+ "revertant" viruses indicated that the temperature-sensitive (*ts*) and attenuation phenotypes specified by the PB2 gene of the *ca* influenza A donor virus were suppressed by a single amino acid substitution in the PA gene that codes for another protein of the polymerase complex.

Construction of an attenuated influenza A virus by introduction of specific mutations into the cDNA of an influenza A virus gene and rescue of the mutant gene into a viable chimeric virus. A chimeric neuraminidase (NA) gene that contained the coding region of the influenza A/WSN/33 NA and the 3' and 5' nontranslated sequences of the nonstructural (NS) gene of the influenza B/Lee virus was introduced into the genome of influenza A/WSN/33 virus by transfection, gene rescue and reassortment. The resulting reassortant virus was highly attenuated for mice and replicated poorly in the upper respiratory tract. Nonetheless, prior infection with the mutant protected mice from virulent influenza A/WSN/33 virus challenge. The virus bearing the chimeric gene, therefore, has many properties that make it desirable for use as a live attenuated vaccine virus and thus it represents the first candidate live attenuated influenza A virus vaccine generated by recombinant DNA technology.

Intragenic suppression of the *ts* phenotype specified by a 12 amino acid deletion in the NS gene. The *ts* phenotype specified by a 12 amino acid (AA) deletion (AA 66-77) in the NS1 protein of influenza A virus was suppressed by a single amino acid substitution of valine for alanine at AA23. The observation that a point mutation can alter the phenotype specified by an appreciable deletion mutation indicates that use of deletion mutation to achieve attenuation does not insure the genetic stability of the resulting virus mutant *in vivo*.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00325-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Respiratory Viruses in Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Brian R. Murphy, M.D.	Head, RV Section	LID, NIAID
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Others:	Peter L. Collins, Ph.D.	Senior Staff Fellow	LID, NIAID
	Susan L. Hall, Ph.D.	Staff Fellow	LID, NIAID
	James Crowe, M.D.	Research Associate (CO)	LID, NIAID
	Robert M. Chanock, M.D.	Chief	LID, NIAID

COOPERATING UNITS (if any)

Sema Laboratories, Rockville, MD (Philips); Georgetown University (London); Wyeth Laboratories (Hung); Marshall University (Belshe); GeneLabs (Larrick)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.45

PROFESSIONAL:

0.7

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Poor immunogenicity of live adenovirus recombinants expressing RSV F glycoprotein that were tested in a susceptible chimpanzee. A single susceptible chimpanzee administered Ad4-F, Ad7-F, and Ad5-F sequentially, by the enteric route, failed to develop appreciable RSV specific antibodies despite efficient replication and immunogenicity of the adenovirus vector.

Cold-passaged viruses derived from a wild-type parainfluenza virus type 3 evaluated in rhesus monkeys, chimpanzees and humans. Cold-passaged (cp) parainfluenza virus type 3 candidate vaccines derived from the JS wild type virus were attenuated, immunogenic and protective in rhesus monkeys. Virus at each of the cold passages tested, i.e., cp12, cp18, and cp45, was attenuated for the upper and lower respiratory tract. The cp45 was the most restricted in the lower respiratory tract, likely due to its greater temperature sensitivity. The attenuating mutations of cp12 and cp18 are non-*ts*. cp45 possesses additional attenuating mutations and these are *ts*. The cp45 retained its *ts* phenotype following replication in rhesus monkeys.

Isolates recovered from (i) chimpanzees administered cp12 virus or (ii) humans administered the cp18 virus lost their *ts* phenotype. Nonetheless, the *ts*⁺ "revertant" viruses retained the attenuation phenotype when evaluated in rhesus monkeys. This indicates that the attenuation phenotype of these cp viruses is stable after replication in primates and this component of the attenuation phenotype is specified by one or more non-*ts* mutations. Furthermore, it appears that the rhesus monkey can be used to assess the attenuation phenotype of isolates from simian or human vaccinees.

Substitution of a single avian influenza A virus gene, PB2, into a wild-type human influenza A virus yields a reassortant that is restricted in both tissue culture and monkeys. A single gene reassortant containing the avian influenza A/Mallard/NY/78 PB2 gene and deriving its other seven RNA segments from the human influenza A/LA/2/87 (H3N2) virus was overattenuated in squirrel monkeys indicating that its restriction of replication in mammalian cells *in vitro* is reflected in its restriction of replication *in vivo*. This restriction appears to be a gene constellation affect, i.e., incompatibility of polymerase proteins derived from avian and human influenza viruses.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00326-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Respiratory Viruses in Volunteers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

Flow Labs, Rockville, MD (Potash); Johns Hopkins University, Baltimore, MD (Clements); Marshall University School of Medicine, Huntington, WV (Belshe)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Parainfluenza Virus Type 3 (PIV3): The JS wild type strain of PIV3, its cold-passaged (cp) cp18 derivative and the bovine PIV3 virus were evaluated in 83 adult volunteers. The candidate vaccines and the parental wild type virus were avirulent and poorly infectious for adults each of whom had been infected previously with PIV3.

The cp18 mutant was evaluated in pediatric subjects 6 to 36 months of age, most of whom possessed serum antibody to PIV3. Nine vaccinees shed PIV3 and two of these children developed evidence of lower respiratory tract disease. In addition, virus was recovered from contacts of the vaccinees. Some of the virus isolates lost their *ts* phenotype, but retained their attenuation phenotype as indicated by studies in monkeys. These results suggest the the cp18 vaccine retains some reactogenicity (detectable only in seronegative vaccinees) and is transmissible. For these reasons future studies will concentrate on the more attenuated cp45 mutant that had undergone 27 additional passages at suboptimal temperature (20°C) in cell culture.

The bovine PIV3 candidate vaccine strain was not shed by seropositive vaccinees and 2 of 4 seronegative vaccinees shed virus without developing symptoms.

Influenza A virus: Six single gene reassortants (SGR) containing an avian influenza A virus PB1, PB2, PA, NP, M, or NS gene in a background of seven other RNA segments of the human influenza A/LA/2/87 (H3N2) wild type virus were evaluated for their level of replication in squirrel monkeys and humans. Dissociation between attenuation for monkeys and humans was observed for the NP and M SGR indicating that studies in monkeys do not necessarily predict the response of humans to these reassortant influenza A viruses. However, concordance between human and monkey virulence was observed for a 6-2 gene (HA and NA genes of human influenza A virus origin) reassortant and for the PB1, PB2, PA, and NS gene SGRs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00327-10 LID
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Laboratory Studies of Parainfluenza Type 3 Virus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Susan L. Hall, Ph.D.	Staff Fellow LID, NIAID
Others:	Brian R. Murphy, M.D.	Head, RV Section LID, NIAID
	Anne Stokes, Ph.D.	Visiting Fellow LID, NIAID
	Robert M. Chanock, M.D.	Chief LID, NIAID
COOPERATING UNITS (if any) 		
LAB/BRANCH Laboratory of Infectious Diseases		
SECTION Respiratory Viruses Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	2.85	PROFESSIONAL: 2.1 OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The PIV3 F glycoprotein expressed by a recombinant baculovirus is antigenically authentic as determined using a panel of PIV3 F specific monoclonal antibodies. Only a low level of antibody was induced by immunization of animals with insect cells infected with the baculovirus F recombinant and the animals were moderately protected against PIV3 challenge. Although the baculovirus expression system appears to be a reasonable source of authentic PIV3 F protein, its poor immunogenicity suggests that its use as a subunit vaccine is problematic.</p> <p>The complete nucleotide sequence of the JS strain of PIV3 was determined and compared to that of the prototype A/Wash/1957 PIV3 strain. Regulatory regions of the genomes were highly conserved, the 3' and 5' non-coding regions showed up to 14% sequence divergence, and the coding regions of all genes other than P showed only 1 to 2% amino acid divergence. A complete cDNA copy of the JS PIV3 wild type is being assembled.</p> <p>The attenuated cold passage 12 mutant of the JS strain of PIV3 was also sequenced and thirteen nucleotide changes that occurred during passage <i>in vitro</i> were identified. The cp12 mutant sustained three nucleotide changes in regulatory regions of the genome and 7 changes in the coding region that lead to amino acid substitution. The nucleotide or amino acid changes responsible for the temperature-sensitive (<i>ts</i>) and attenuation (<i>att</i>) phenotypes remain to be determined.</p> <p>A technique for sequence analysis of a hypervariable region of the 5' non-coding region of the F gene has been developed that can identify PIV3 isolates from vaccinees as vaccine-derived or community-derived virus. This will be especially helpful in identifying the origin of isolates that have lost one or more phenotypes of a candidate vaccine virus (such as the <i>ts</i> property) following replication in vaccinees.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00345-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Laboratory Studies of Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brian R. Murphy, M.D. Head, RV Section LID, NIAID

Others: Robert M. Chanock, M.D. Chief LID, NIAID
Peter Collins, Ph.D. Microbiologist LID, NIAID
Mark Connors, M.D. Research Associate (CO) LID, NIAID
James Crowe Research Associate LID, NIAID
Arun Kulkarni Visiting Fellow LID, NIAID

COOPERATING UNITS (if any)

Upjohn Company, Kalamazoo, MI (Wathen), LIP/NIAID/NIH (Morse)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.15

PROFESSIONAL:

3.15

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cotton rats previously immunized with a chimeric FG glycoprotein of human respiratory syncytial virus (RSV) developed enhanced pulmonary histopathology following challenge with RSV. This potentiation of pathology was not observed in animals previously immunized with an adenovirus-F or vaccinia-F recombinant. Unlike RSV infection or infection by an adenovirus-F or vaccinia virus-F recombinant which each induced a moderate to high level of serum RSV neutralizing antibodies, immunization with RSV FG induced a low titer of serum RSV neutralizing antibodies although the total quantity of antibodies produced was very high. As a consequence, FG immunized cotton rats were not resistant to RSV challenge. These abundant "low quality" antibodies do not appear to be responsible for enhanced pulmonary pathology during subsequent RSV infection because passive transfer of post-immunization sera to other animals did not result in enhancement of lung lesions during RSV infection. This suggests that enhanced histopathology is mediated by RSV-specific T-cells.

Enhanced pulmonary histopathology was also observed in mice previously immunized with formalin-inactivated (FI) vaccine. FI RSV-immunized mice depleted of CD4 T-cells prior to RSV challenge, but not those depleted of CD8 T-cells, failed to develop enhanced pulmonary histopathology indicating that CD4 T-cells were the mediators of enhanced pulmonary histopathology.

During a study in which mice were immunized with one of a series of vaccinia-RSV recombinant viruses that separately encode a single RSV protein, it was observed that the F, G, N, and M2 proteins each induced resistance to RSV challenge, but the resistance induced by N and M2 was relatively short-lived. This study suggested that RSV vaccines need only contain F and G glycoproteins, because the other RSV proteins either fail to induce resistance or induce immunity that is less effective and more transient than the resistance provided by the F and G antigens. The resistance induced by M2 was shown to be mediated by CD8 T-cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00368-09 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of Respiratory Syncytial Virus (RSV) Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph.D. Senior Staff Fellow LID, NIAID

Others: David S. Stec, Ph.D. NRC Fellow LID, NIAID
Michael A. Mink, Ph.D. IRTA LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.25

PROFESSIONAL:

1.75

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are exploring methods of obtaining biological activity for synthetic RSV vRNA made from cDNA clones.

In one approach, we have constructed cDNAs which encode truncated, ca. 1000-nucleotide "mini" vRNAs. These contain marker genes, such as the chloramphenicol acetyl transferase (CAT) gene, under the control of RSV transcriptive signals and flanked by the RSV 3' and 5' noncoding vRNA termini. The plan is to transfect the "mini" vRNAs into tissue culture cells and superinfect with RSV to provide helper proteins to drive their replication and expression. This would provide a system for (i) performing structure-function studies of cis-acting RNA sequences and (ii) identifying and characterizing the viral proteins involved in RNA transcription and replication. In preliminary experiments, we have been able to show that transfection of two different vRNA analogs into cells results in the appearance of CAT activity dependent upon superinfection with RSV. But the success rate in these early experiments is 50%. The efficiency and success rate probably can be improved by optimizing the experimental conditions, and the fact that some success has been obtained suggests that this can be developed into a feasible experimental approach.

A second line of experiments had been to construct a complete cDNA of the 15,222-nucleotide vRNA which will be used to produce ("rescue") live RSV. This would be a method for introducing defined genetic changes into RSV for molecular studies and as an approach to characterizing existing attenuated strains and producing new strains that could be used as vaccines. Because existing cDNAs were synthesized from mRNA, it has necessary to reclone most of the vRNA in order to insert intergenic sequences and, in some areas, new restriction which will be useful in construction of subsequent mutants. Most of the vRNA has been recloned, and the complete cDNA is under construction.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00372-09 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

RSV Proteins: Roles in Host Immunity and Immunoprophylaxis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph.D. Senior Staff Fellow LID, NIAID

Others: Geoffrey Cole, Ph.D. IRTA LID, NIAID
 Mark Connors, M.D. Research Associate (CO) LID, NIAID
 Robert M. Chanock, M.D. Chief LID, NIAID
 Brian R. Murphy, M.D. Head, RV Section LID, NIAID

COOPERATING UNITS (if any)

Upjohn Company, Kalamazoo, MI (Nicholas)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.75

PROFESSIONAL:

1.25

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are investigating approaches for utilizing recombinant DNA techniques for developing subunit vaccines for human respiratory syncytial virus (RSV). (An accompanying report describes efforts to use recombinant DNA technology to improve attenuated RSV vaccine strains.)

We have been employing a panel of vaccinia recombinants expressing individually nine of the ten major RSV proteins to explore the roles of the different proteins in inducing antibodies and cytotoxic T cells (CTL), primarily in the BALB/c mouse. The issues of antibody induction, protective immunity and disease enhancement are described more thoroughly in an accompanying report (Murphy *et al.*, accompanying report). A recombinant expressing the 22K or M2 protein (vac-M2) induced levels of CTL which were comparable to those induced by intranasal RSV infection. Vac-M2 induced significant levels of protective immunity in the absence of a detectable antibody response. This indicated that CTL alone can restrict the replication of RSV, a finding which had not been described previously for a virus which replicates in the respiratory tract.

The use of adenovirus as a viral vector offers several potential advantages for RSV immunoprophylaxis. Previously, we described the construction of an adenovirus type 5 recombinant which expresses the F glycoprotein. The ad-F recombinant was highly immunogenic and protective in cotton rats (annual report, 1989, 1990) and mice (Murphy *et al.*, accompanying report). However, ad-F was not effective in inducing RSV-specific serum antibodies in chimpanzees (Murphy *et al.*, accompanying report). The possibility exists that the replacement of adenovirus sequences by the F gene altered the replication, expression or immunogenicity of the vector. Therefore, we are constructing a series of recombinants which contain insertion sites which are engineered to avoid the deletion of adenovirus sequences and to minimize alteration of adenoviral replication and gene expression.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00498-05 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis, Processing and Functions of the Proteins of Human RSV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter L. Collins, Ph.D. Senior Staff Fellow LID, NIAID

Others: David S. Stec, Ph.D. NRC Fellow LID, NIAID
Genevieve Mottet Special Volunteer LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

0.75

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In previous work, we showed that RSV encodes three transmembrane glycoproteins, the fusion F glycoprotein involved in viral penetration, the attachment G glycoprotein, and the small hydrophobic SH protein of unknown function. The post-translational processing of the three proteins was studied in parallel. Inhibitors of exocytosis were employed to identify intermediates in processing and to operationally define the intracellular sites of processing steps such as oligomerization, palmitoylation, poly lactosaminylation, cleavage of the F protein and O-glycosylation of the G protein. Sucrose gradient sedimentation and chemical cross-linking were used to monitor oligomerization, and lectin-binding and endoglycosidases were used to monitor O-glycosylation. One finding was that the oligomerization of the G protein occurs in the endoplasmic reticulum, whereas its O-glycosylation does not occur until the trans Golgi compartment. This implies that the O-linked sugars are not important determinants of oligomerization and, by implication, of polypeptide folding.

Studies are continuing to analyze the multiple forms and complex processing scheme of the SH protein using site-directed mutagenesis and biochemical techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00308-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vitro and *In Vivo* Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

Others: Stephen M. Feinstone, M.D. Medical Officer LID, NIAID

COOPERATING UNITS (if any)

Smith Kline RIT (d'Hondt); FDA (Daemer); Fairfield Hospital, Melbourne, Australia (Gust); AFIP (Ishak); WRAIR (Sjogren)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INACTIVE 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00311-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Search For New Hepatitis Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

Others: Patrizia Farci, M.D. Visiting Scientist LID, NIAID

COOPERATING UNITS (if any)

Hospital for Sick Children, Toronto, Canada (Phillips); Howard University Medical School (Sewchand)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

In 1989 a possible paramyxovirus etiology for giant cell hepatitis was proposed, based on electron micrographic changes seen in the livers of patients with this disease. Collaborative studies, including attempts to transmit the disease to primates, are in progress.

Patients with thalassemia in Sardinia, Italy, receive monthly blood transfusions as therapy. Many of these patients have developed chronic hepatitis. Most cases could be identified as hepatitis B or hepatitis C, but some lacked markers of either of these viruses. These patients are being studied for evidence of previously unrecognized hepatitis viruses.

The objectives of this project are to identify and characterize new etiologic agents of hepatitis and to develop useful assays for diagnosis of infection and seroepidemiologic studies. A longer term objective is the development of passive and active immunoprophylaxis for these important human pathogens.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00314-10 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Woodchuck Virus: Molecular Biological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Roger Miller, Ph.D Senior Staff Fellow LID, NIAID

Others: Cathie Chung, Ph.D Senior Staff Fellow LID, NIAID
Hong-shu Chen, M.D. Visiting Fellow LID, NIAID
Michael Kew, M.D., Ph.D. Visiting Scientist LID, NIAID
Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

Division of Molecular Virology & Immunology, Georgetown University, Washington, DC (J Gerin);
New York State College of Veterinary Medicine (Tennant)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.7

PROFESSIONAL:

1.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts and these associations appear to be etiological in nature. Thus, WHV infection of woodchucks provides a relevant and convenient model for understanding HBV infections of humans. The following experiments were performed: (1) The variability of WHV genomes in an infected animal was determined. (2) Serum pools with and without defective virus were found to induce the same percentage of chronic carriers. This suggests that the presence of defective virus in a WHV inoculum is not a prerequisite for the establishment of persistent hepadnavirus infections. (3) Oligonucleotide directed mutagenesis was used to introduce point mutations in the precore and X genes as well as open reading frames 5 and 6 to determine the importance of these genes in virus replication using woodchuck transfection assays. (4) The WHV and HBV X gene promoters were cloned into the vector pSV0CAT to compare promoter strength in human and woodchuck cells. (5) A New York strain of WHV was cloned in preparation for DNA sequencing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00530-04 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Computer Analysis of the Hepatitis B Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Roger H. Miller, Ph.D. Senior Staff Fellow LID, NIAID

Others: Shuichi Kaneko, M.D. Visiting Fellow LID, NIAID
Robert H. Purcell, M.D. Head, HVS LID, NIAID

COOPERATING UNITS (if any)

Liver Diseases Section, National Institutes of Diabetes and Digestive and Kidney Diseases (Baker, Di Bisceglie, Hoofnagle)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The technique of amplifying DNA using the polymerase chain reaction (PCR) assay is a powerful method for detecting low levels of DNA. We developed a simple and rapid PCR method for the detection of hepatitis B virus (HBV) DNA in serum. In testing serial samples from five chimpanzees experimentally infected with HBV, we found that HBV DNA was detected 2-3 weeks before the appearance of hepatitis B surface antigen (HBsAg), and continued to be detectable 1-3 weeks after the production of antibody to HBsAg. Sera from 98 patients at various stages of chronic HBV infection were also studied. Patients were divided into three groups according to their HBsAg and hepatitis Be antigen (HBeAg) status. Group I patients were positive for both HBsAg and HBeAg in the serum. PCR analysis demonstrated that all 31 (100%) of these patients possessed serum HBV DNA. Group II patients were positive for HBsAg and negative for HBeAg. Analysis showed that 36 of 46 (78%) of these patients possessed serum HBV DNA. Group III patients were former chronic HBV carriers but subsequently lost HBsAg during follow up. In this group 5 of 21 (24%) were found to harbor HBV DNA sequences in the serum. Detection of HBV DNA by PCR analysis is a valuable method for detecting the presence of DNA-containing virions in serum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00555-03 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Detection of Hepatitis B Virus DNA Using the Polymerase Chain Reaction Assay

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Roger H. Miller, Ph.D. Senior Staff Fellow LID, NIAID

Others: Shuichi Kaneko, M.D. Visiting Fellow LID, NIAID
Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

Liver Diseases Section, National Institutes of Diabetes and Digestive and Kidney Diseases (Di Bisceglie, Hoofnagle)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

TERMINATED 1990-91

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00569-02 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of Genetic Markers of Virulence and Adaptation to Cell-Culture of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Suzanne U. Emerson, Ph.D. Microbiologist LID, NIAID

Others: Sergei Tsarev, Ph.D. Visiting Fellow LID, NIAID
Susan Gdovin, Ph.D. Staff Fellow LID, NIAID
Valeria Tedeschi, Ph.D. Visiting Scientist LID, NIAID
Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis A virus (HAV) is a picornavirus with a single-stranded RNA genome of approximately 7500 nucleotides. The wild-type strain of HAV grows poorly in cell-culture, is not cytopathic, and yields a low titer of virus. A cell-culture adapted mutant has been isolated which grows significantly more efficiently in cell-culture and which is attenuated for marmosets and chimpanzees. The objectives of this project are to determine the genetic basis for virulence and adaptation to cell culture of HAV.

1. The molecular basis of the ability of attenuated HAV strain, HM-175, to grow in cell culture has been determined. Growth in cell culture is dependent upon three mutations in the 2B and 2C regions of the HAV genome. Host cell specificity is conferred by changes in the 5' noncoding region.

2. A deletion in the non-infectious cDNA clone containing the genome of wild-type virus was identified and corrected and the new cDNA clone was shown to be infectious for cultured cells and to produce virus that caused severe hepatitis in marmosets.

3. Simian strain AGM-27 was recovered from an infected, ill African green monkey. This HAV strain was evaluated for host range and virulence in several primate species. It is attenuated for chimpanzees but not African green monkeys or marmosets and is being further evaluated as a candidate live hepatitis A vaccine. It has been almost entirely sequenced and found to be significantly different from human HAV strains.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00570-02 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Hepatitis C Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Roger H. Miller, Ph.D.	Senior Staff Fellow	LID, NIAID
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Others:	Robert H. Purcell, M.D.	Head, HVS	LID, NIAID
	Patrizia Farci, M.D.	Visiting Scientist	LID, NIAID
	Norio Ogata, M.D., Ph.D.	Visiting Associate	LID, NIAID
	Jens Bukh, M.D.	Visiting Associate	LID, NIAID

COOPERATING UNITS (if any)

National Institutes of Health, Japan (Shimizu); Chiron Corporation (Weiner)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

2.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis C virus (HCV) is an important human pathogen that is strongly associated with transfusion related non-A, non-B (NANB) hepatitis. Patient H is a patient who has been a chronic HCV carrier for the past 13 years. We compared the nucleotide and predicted amino acid sequences of the HCV genome obtained from plasma collected in 1977 with that collected in 1990 and found that the two HCV isolates differ at 123 of the 4,923 (2.50%) nucleotides sequenced. We estimate that the mutation rate of the H strain of HCV is approximately 1.9×10^{-3} base substitutions per genome site per year. The nucleotide changes were exclusively base substitutions and were unevenly distributed throughout the genome with a relatively high rate of change observed in the nonstructural protein number 1-like gene region. Our results suggest that the mutation rate of the HCV genome is similar to that of other RNA viruses and that HCV genes appear to be evolving at different rates within the virus genome.

Although the development of a serologic assay to detect antibody to HCV has greatly extended our knowledge of NANB hepatitis, little is known about the course of viremia during HCV infection. Therefore, we investigated the pattern of serum HCV RNA using the polymerase chain reaction assay and its relationship to antibody response and clinical outcome in the course of NANB hepatitis. Serum HCV RNA first appeared within 1 week post-infection in the majority of patients examined preceding the clinical onset of hepatitis and antibody seroconversion. Viremia was transient, lasting only a few months in acute self-limited hepatitis, but persisted up to 14 years in patients chronically infected. Serum HCV RNA may provide prognostic information regarding progression of acute to chronic virus infection.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00596-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vivo and *In Vitro* Studies of Hepatitis E Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Suzanne U. Emerson, Ph.D. Microbiologist LID, NIAID

Others: Sergei Tsarev, Ph.D. Visiting Fellow LID, NIAID
Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any)

National Institute of Virology, Pune, India (Arankalle); All India Institute of Medical Research, New Delhi, India (Panda); Gene Labs, Inc., Redwood City, CA (Reyes); WRAIR, Washington, DC (Sjogren, Ticehurst); USUHS, Bethesda, MD (Letgers)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to obtain an increased understanding of hepatitis E virus (HEV) and related viruses with the goal of designing strategies for their control. Although sequence analysis of HEV clones is still rather limited, preliminary data indicate that this virus, like most single-stranded RNA-containing viruses, exhibits significant heterogeneity of genomic sequence. This has potential implications for vaccine development, since heterogeneity of the degree exhibited by this virus is sometimes associated with serotypic variation. However, the limited data available on cross-challenges in primate models suggest that even relatively divergent strains will elicit cross-protective antibodies.

1. A standard challenge pool of hepatitis E virus has been prepared from an acute-phase stool specimen obtained from an epidemic of hepatitis E in Sargodha, Pakistan. This pool has been biologically characterized in several primate species.

2. Reagents have been developed that are useful for the detection of HEV and for evaluation of newly emerging serologic tests.

3. The SAR-55 strain of HEV is being cloned and sequenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00370-09 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Simian Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert Olmsted, Ph.D. Senior Staff Fellow LID, NIAID

Others: Simoy Goldstein, Ph.D. Expert LID, NIAID
Robert M. Chanock, M.D. Chief LID, NIAID

COOPERATING UNITS (if any)

LIG, NIAID, NIH, Bethesda, MD (Fauci, Koenig); Georgetown University (Formsgard, Gerin, Hirsch, Johnson, London); Delta Primate Center (Murphy-Corb); Southwest Foundation (Allen)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

6.0

PROFESSIONAL:

2.0

OTHER:

4.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major goal of this Project is to establish an animal model for human AIDS in which to study viral determinants of immunodeficiency and immunologic correlates of resistance to infection or disease. Simian immunodeficiency virus (SIV) isolated from sooty mangabey monkeys (SIVsm) readily induces an immunodeficiency syndrome in macaques similar to human AIDS, therefore this strain has been the focus of pathogenesis and vaccine studies.

The genetic analysis of African strains has focused on SIVsyk recovered from a sykes monkey and SIV isolated from the tantalus sub-species of African green monkey (AGM). Sequence analysis of the sykes isolate indicated that it is a distinct new member of the primate lentiviruses. The tantalus strain groups with other African green monkey SIVs but forms its own species-specific subgroup.

Pathogenesis studies have addressed: (i) the molecular pathology of end-stage SIVsm-induced immunodeficiency; and (ii) the extent and rate of genetic drift of SIVsm molecular clones. High levels of unintegrated viral DNA were observed in most tissues. Virus existed as a swarm of related genomes and resided mainly within macrophages. Ten distinctive (in terms of sequence and tropism) infectious clones were obtained directly from the spleen of one of these macaques. Progeny virions of one of these clones induced a persistent decline in circulating CD4 lymphocytes within 6 months of infection. Analysis of genetic drift of SIVsm molecular clones demonstrated that SIV undergoes significant variation upon replication *in vivo*; the variation is far greater in the envelope gene than within the integrase gene. Finally, full-length infectious molecular clones of SIVstm from stump-tail macaques and SIVsm/PBj, the acutely lethal variant, were generated. The SIVstm clone groups with other reported SIVmac clones but is genetically distinct. Virus stocks generated from two PBj clones (sharing a common 3' end) induced the typical PBj syndrome of acute early death.

Finally, an inactivated whole SIV vaccine was tested in macaques. Vaccinated monkeys developed a strong SIV antibody response and were protected from challenge with cell-free virus of an heterologous SIV strain as well as the homologous SIV strain used to produce the vaccine. The vaccinated animals will be challenged with cell-associated virus in the near future.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00616-01 LID

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Feline Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert Olmsted, Ph.D. Senior Staff Fellow LID, NIAID

Others: Raymond Langley, Ph.D. Visiting Associate LID, NIAID

Diane Adger-Johnson Biologist LID, NIAID

Robert Goeken Biologist LID, NIAID

COOPERATING UNITS (if any)

Georgetown University (Johnson, Hirsch, Goff, Albert); NCI (O'Brien); Florida Fresh Water Fish and Game Commission (Roelke); Center for Retroviral Research, Ohio State University (Lafrado)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

1.5

OTHER:

2

CHECK APPROPRIATE BOX(ES)

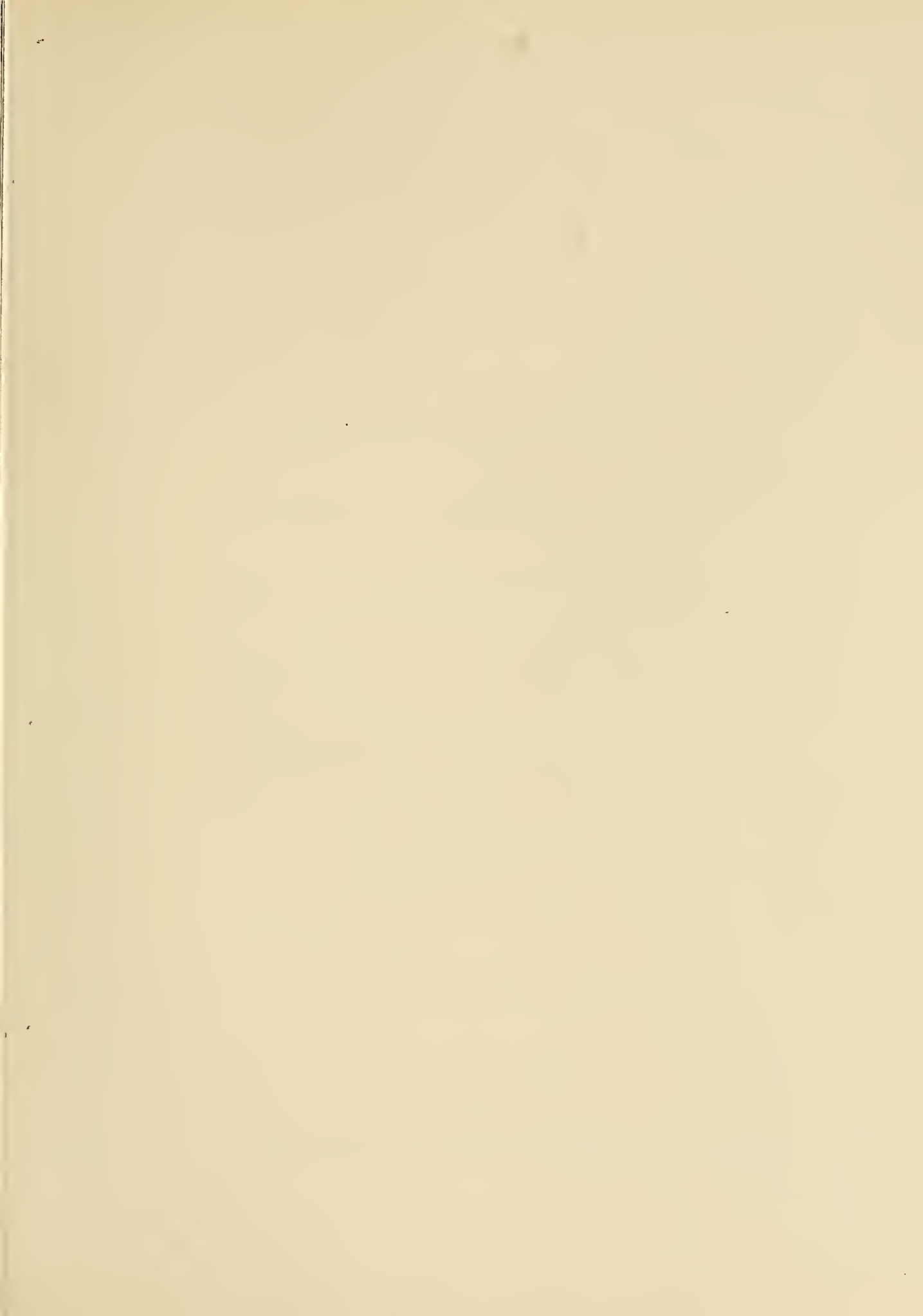
☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To date, experimental infection of cats with the California prototype strain of FIV has produced little if any disease. Two approaches were undertaken to identify isolates of FIV or FIV-related lentiviruses that might prove useful in the development of an experimental cat model for AIDS. Strain FIV-MA was isolated from a clinically-ill two year old domestic cat in Maryland and was used to inoculate SPF weanling kittens. Unlike our previous experience with experimental FIV infection of SPF kittens, the FIV-MA infected animals failed to gain weight at a normal rate and at five weeks post-infection (p.i.) the animals displayed symptoms of a neurological disorder characterized by "compulsive roaming" behavior. Preliminary neurological examination suggested that the affected kittens had progressed to an early stage of encephalopathy.

771 serum/plasma samples from North American and African free-ranging and captive felids were screened by FIV immunoblot for serological evidence of feline lentiviral infection. Cross-reactive antibodies to FIV were detected with highest frequency in puma/Florida panther, East and South African lion and East African cheetah populations. Novel isolates of feline lentiviruses (puma lentivirus-PLV) were obtained by PBL coculture from several free-ranging Florida panthers (*Felis concolor coryii*). Sequence comparison of the PLV-RT gene with FIV showed a significant degree of diversity between the two feline lentiviruses.

Sequence alignment of the *env* gene products of five FIV isolates from different geographic locations revealed variable and conserved regions similar to the primate lentiviruses.



LABORATORY OF MOLECULAR MICROBIOLOGY
1990 Annual Reports

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LABORATORY OF MOLECULAR MICROBIOLOGY

SUMMARY

The Laboratory of Molecular Microbiology (LMM) applies molecular and biological techniques to study the structure, function, and regulation of prokaryotic and eukaryotic genes. Programmatically, murine retroviruses, the human immunodeficiency virus (HIV) and human T-cell leukemia virus type 1 (HTLV-1), and mycoplasmas and their host cells are investigated. Members of the LMM are physically located in Building 4 on the Bethesda campus and in Building 550 at the Frederick Cancer Research Facility. Although the research activities conducted by the LMM staff are quite diverse, a common theme involves the characterization of genetic elements (both chromosomal and episomal) affecting the interaction of microorganisms and the cells they infect.

STUDIES OF THE HUMAN IMMUNODEFICIENCY VIRUS

Molecular biological and virological techniques have been employed to dissect structure/function relationships of structural and regulatory genes of the human immunodeficiency virus (HIV) during acute and chronic virus infections. In contrast to most other groups, this laboratory has tended to focus on the effects of mutated viral genes evaluated in the context of the complete viral genome; complementary *in vitro* analyses of isolated HIV genes have also been carried out.

Structural and functional analysis of the HIV-1 Vpu gene product. The HIV-encoded Vpu protein is an integral membrane phosphoprotein which is present in high concentrations in virus-producing cells but appears to be excluded from virus particles. Vpu forms homopolymeric complexes *in vivo* and *in vitro*; protease protection studies indicate that Vpu possesses the topology of a Class I integral membrane protein but lacks the signal peptide processing signal. An analysis of potential interactions of Vpu with other viral or cellular proteins demonstrated that it affected the processing of the HIV envelope glycoprotein precursor gp160 and the intracellular stability of CD4. In the absence of Vpu, gp160 and CD4 form stable intracellular complexes which cannot be transported intracellularly. This blocks the maturation and cell surface expression of both proteins and leads to a reduction in the production of progeny virions. In the presence of Vpu, gp160/CD4 complexes are destabilized and normal synthesis of gp120/gp41 ensues. In other experiments, the steady-state levels of CD4 have been shown to be reduced in the presence of Vpu. Thus Vpu has a bimodal effect on HIV production: 1) it causes the dissociation of gp160/CD4 complexes and 2) it induces the degradation of "free" CD4.

(Strebel, Maldarelli, Martin, and Willey).

Identification of inhibitory sequences (IR elements) within the HIV genomic RNA A sensitive test system, which employs the HIV *tat* gene, has been developed to identify sequences present in the viral genome which inhibit the utilization of HIV mRNAs. Sequences encoding Gag and

Pol gene products were cloned downstream of *tat* and their inhibitory effect on Tat expression was monitored in a chloramphenicol acetyl transferase (CAT) indicator cell line. Two independent elements were identified which severely blocked the synthesis of Tat protein: one located in the *gag* and one mapping to the *pol* genes. The inhibitory effect of these elements was verified in a heterologous system and found to be "orientation" dependent. The presence of the negative elements resulted in the accumulation of spliced and unspliced transcripts in the nucleus of transfected cells. Thus the inhibition of gene expression by IR elements appears to occur at a post-transcriptional level and results in a dramatic reduction of IR-containing transcripts in the cytoplasm of transfected cells. Studies are in progress to analyze the role of IR elements in the Rev-dependent regulation of HIV gene expression.

(Maldarell, Martin and Strebel).

Mutations in the HIV *env* gene affect the intracellular transport and processing of gp160. Intracellular transport and processing of the HIV envelope precursor polyprotein gp160 proceeds via the endoplasmic reticulum (ER) and Golgi complex. Results from pulse-chase analyses indicated that a single amino acid substitution within a highly conserved domain of the *env* gene impaired gp160 export from the ER, leading to an increase in oligomeric forms of gp160 and a decrease in gp120 production. In contrast, a gp160 gene which contained a mutated cleavage site, encoded a protein which was able to traverse the ER/Golgi complex, even in the absence of proteolytic processing, and became incorporated into progeny virions. These results indicate that export from the ER is a point in the intracellular trafficking of gp160 which is critical in the production of mature envelope components.

(Willey and Martin)

Analysis of the biochemical function of Vif from HIV-1 and HIV-2. The HIV-1 and HIV-2 *vif* genes were amplified by the polymerase chain reaction (PCR) and cloned into plasmid vectors for sequencing. These genes were subsequently inserted into T7 expression plasmids for protein production in *E. coli* and into a plasmid containing the human CMV promoter for expression in mammalian cells. Using the T7 expression system, HIV_{LAI} Vif was produced and injected into rabbits for the production of specific antibodies. Unfortunately, the HIV-2 Vif protein produced in the prokaryotic system is unstable. In an attempt to overcome this latter problem, Vif fusion proteins will be expressed in *E. coli* and tested for increased stability. Several HeLa cell lines have been derived in which the *vif* of HIV-1 and HIV-2, under the control of the CMV promoter, are currently being characterized.

(Fan and Peden)

Characterization of infectious molecular clones of HIV_{LAI}, MAL and ELI. Full length infectious molecular clones of the LAI (formally called BRU), MAL and ELI derivatives of HIV-1 were assembled. Viruses derived from all three clones were found to be infectious in peripheral blood mononuclear cells (PBMC), with LAI and ELI inducing syncytia in the cultures while MAL did not. The spectrum of tropism on different cell lines was found to be distinctive for each virus. LAI could replicate in all T cell lines tested (CEM, H9, SupT1, Jurkat, MT-4) and a promonocyte cell line (U937). MAL was only infectious in SupT1 cells. ELI was able to grow well in MT-4 and Jurkat cells but exhibited delayed replication kinetics in CEM and H9 cells, and was unable

to infect U937 cells. HIV_{ELI}, propagated in H9 cells, was used to infect CEM and H9 cells; interestingly, the viral progeny no longer exhibited delayed growth kinetics and was also able to infect U937 cells which had been refractory to infection with the parental virus. These results demonstrate that the phenotype of certain HIV strains is not stable to passage in tissue culture, and both the replicative capacity and tropism can be altered by adaptation to growth in different cell lines.

(Peden)

Changes in HIV-1 associated with adaptation to growth in tissue culture cell lines. Virus derived from the infectious molecular clone corresponding to HIV_{ELI}, which grows poorly in the H9 T-cells and not at all in the U937 promonocyte cell line (see above), can be adapted to grow in U937 cells following passage in the H9 line. The genome of the adapted virus was compared to that of the parental clone using PCR and a technique called single strand conformational polymorphism (SSCP), which is sensitive to single base changes in the pool of amplified DNA. Two regions in the envelope gene where sequence changes occurred upon passage in H9 cells were identified and these alterations were shown to confer the enhanced growth phenotype when incorporated into the parental virus. The location of the mutations suggests that altered binding to the CD4 receptor and altered fusing capacity subsequent to adsorption may be involved in adaptation to growth in cell lines.

(Fujita, Silver and Peden)

Reconstructed HIV long terminal repeats confer altered replicative capacity in human T lymphocytes. Because of the importance of retroviral long terminal repeats (LTRs) as determinants of tropism and pathogenic potential, *in vitro* mutagenesis techniques have been used to alter these elements in the context of an infectious molecular clones of the human immunodeficiency virus (HIV). Starting with a replication-incompetent molecular clone of HIV lacking the two NFκB and three Sp1 binding sites present in wild-type LTRs, proviruses containing reconstructed LTRs with individual or combinations of NFκB and Sp1 elements were generated and evaluated for their capacity to produce virus progeny following transfection-cocultivation. The virus stocks obtained exhibited a continuum of replicative capacities in different human T-cell types depending on which element(s) was present in the LTR. Of particular interest was the emergence of second-site revertants containing changes affecting the TATA box.

(Ross, Englund and Martin)

The effect of heterologous enhancer elements on the ability of HIV to replicate in mammalian cells. The replication of HIV in mouse cells following the transfection of full-length or subgenomic clones is quite inefficient. For example, the expression of the p24 Gag protein in mouse cells is 5000-fold lower in murine compared to human cells. The HIV LTR was altered by deleting NFκB and/or Sp1 sites and substituting enhancers from CMV, Moloney murine leukemia virus or polyoma virus; such cloned proviral DNAs exhibited higher basal levels of expression in mouse cells but replicative capacity was still severely compromised. These viruses, containing chimeric LTRs, exhibit varied tropism following infection of different human T-cell lines. An attempt is being made to identify and clone factors present in human cells required for high levels of HIV expression in murine cells. As a first step, human/mouse somatic cell hybrids

are being screened for their ability to support vigorous HIV replication.

(Chang and Martin)

Cell-to-cell spread of HIV. An efficient system to monitor the cell-to-cell spread of HIV was developed employing chronically infected H9 donor cells. Under appropriate conditions of co-cultivation, the synthesis of unintegrated viral DNA, monitored by Southern blot hybridization, occurred between 2 and 4 hrs. following infection; viral proteins were detected 8 to 12 hrs following co-cultivation and progeny virions were released into the medium by 16 hrs. The use of metabolic inhibitors and/or specific antibodies revealed that the cell-to-cell spread of HIV required: 1) the interaction of gp120 with the CD4 receptor and 2) reverse transcription. Light and electron microscopy indicated that cell-cell fusion, occurring within 15 minutes of co-cultivation, mediated the rapid spread of the infection. Unexpectedly, the fusion observed did not appear to involve budding or free virus particles.

(Sato and Martin)

Construction of an HIV genome which can be efficiently expressed in mouse cells. The 5' LTR in two HIV-1 isolates was deleted from an infectious molecular proviral DNA clone and replaced with transcriptional regulatory elements from either simian cytomegalovirus (CMV) or Moloney murine leukemia virus. Relative to wild type HIV, increased levels of viral proteins, as evidenced by p24 production, were obtained in mouse cells from the constructs with a heterologous enhancer-promoter region. Constructs containing CMV sequences gave the highest level of expression, even in human cells; assays monitoring extracellular release of reverse transcriptase indicated that the recombinant particles were not infectious, as predicted. Transgenic mice containing the recombinant proviral genomes are now being generated with the aim of establishing a better animal model for HIV induced pathogenesis.

(Mounts and Martin)

UV light activation of HIV LTR driven expression in transgenic mice. Previous experiments demonstrated elevated levels of enzyme activity of CAT in transgenic mice harboring an HIV-LTR driven CAT gene. To investigate if UV was acting at the level of transcriptional activation, attempts were made to measure the amount of CAT RNA using sensitive and quantitative assays. Even in mice made homozygous for the transgene, no CAT RNA could be measured indicating that the level of transcription is very low and quantitative PCR will be required for detection. The antioxidant, sodium diethyldithiocarbamate, is now being tested for its ability to block the UV activation of the HIV LTR in transgenic mice, since thiol compounds have been reported to modulate replication of the virus *in vitro*.

(Mounts and Martin)

HIV-1 Rev-RRE Interactions. The replication of HIV is dependent on the function of the Rev protein which acts post-transcriptionally to modulate the splicing, extra-nuclear transport and/or translational utilization of unspliced (Gag-Pol) or partially (Env) spliced viral RNAs. Rev mediates this function by interacting with a highly structured viral mRNA sequence, the RRE (Rev responsive element) consisting of 236 nt. located in the Env open reading frame. Studies involving approximately 80 RRE mutants have demonstrated that: i) Rev binding to RRE RNA is necessary but not sufficient for Rev function *in vivo*; ii) Rev binding to RRE is dependent on

the presence of a 5'(nt50)CACUAUGGG(nt58)3' sequence in the context of an unique secondary structure; iii) in the nt56..GGG..nt58 sequence, the G-56 is critical and a minimum of 2 Gs are required for RRE function; and iv) mutations distal to the 3 Gs, which cause base-pairing of the Gs, eliminate binding. Rev binding specifically shielded at least two domains on the RRE RNA from nuclease attack. The 5' domain included the above 3 Gs and the 3' domain was mapped between residues 80-87.

(Holland, Park and Venkatesan).

HIV-1 Rev activates heterologous MS2 RNA if tethered to it by MS2 coat protein. A chimeric plasmid was constructed which replaced the Rev binding subdomain of RRE with the RNA phage MS2 translational operator sequence element. This chimeric reporter plasmid, referred to as Gag-RREZMS2, was transactivated by a fusion protein linking the MS2 coat protein to the C-terminus of Rev (Rev-MS2) but not by Rev or MS2 coat protein. A Gag-RRE plasmid in which the MS2 sequence substituted for RRE did not respond to Rev-MS2, implying that other domains in RRE may be required for the binding of a cellular factor. However, a MS2-containing Gag plasmid partially responded to Rev-MS2 if the MS2 sequence embedded within a secondary structure motif that included the stem A of RRE. Genetic studies have identified several important subdomains within the 116 amino-acid Rev protein. These include an activation domain between residues 65-80, an RNA binding/nucleolar targeting domain between amino acids 35-50 and protein oligomerization domains flanking the RNA binding region.

(Venkatesan, Holland and Park)

Biochemical and biological comparison of HIV-1 Nef and Ras proteins. To determine whether GTP binding and GTPase are general properties of Nef proteins, four different Nefs and two different p21 Ras proteins expressed in *E. coli*, were purified. In contrast to Ras, the Nef protein preparations exhibited no detectable GTP binding. However, purified Nef proteins were phosphorylated when incubated in the presence of either GTP or ATP. This putative autokinase activity was higher in Nef proteins containing threonine at position 15 than in those carrying alanine at that site. Two different Nef genes also failed to induce oncogenic transformation of permanently transfected NIH 3T3 cells under conditions that led to oncogenic transformation using activated *ras* genes. Also, unlike *ras*, the Nef gene products failed to induce meiotic maturation when injected into fully-grown *Xenopus* oocytes.

(Nebreda, Wingfield, Venkatesan and Santos).

HIV particle formation. Using a vaccinia virus expression system, p55 Gag protein was synthesized and self-assembled into immature particles which contained a concentric ring of electron dense material. Expression of both Gag and Pol proteins resulted in mature particles containing a condensed nucleoid structure characteristic of lentiviruses. When the *pol* gene was truncated at the end of the protease domain, Gag processing was markedly reduced and no mature particles were observed. Gag processing and particle maturation were virtually absent when the protease was co-expressed in *trans* from a separate vaccinia recombinant. However, extracts

from the protease expressing cells processed denatured p55 Gag protein expressed in *E. coli* and readily hydrolyzed synthetic substrates. This result suggested that either the viral protease cannot enter nascent particles, or more likely, Gag oligomerization is compartmentalized and thus sequestered from protease digestion.

(Ross, Fuerst, Orenstein, Martin and Venkatesan)

Characterization of the HIV-1 LTR target for Tat. The contributory role of the HIV-1 TATAA and enhancers for basal and Tat-induced transcription were analyzed. The minimal HIV-1 promoter, which directs basal levels of expression, was mapped within sequences spanning -43 to +80. Expression directed by this minimal promoter was boosted more by the presence of NF κ B than by Sp1 elements. Although the minimal LTR promoter (-43 to +80), contained an intact TAR sequence, it was not Tat inducible. However, the addition of short synthetic enhancer motifs (AP1, Oct, Sp1, NF κ B) conferred Tat-responsiveness. This ability to respond to Tat was dependent upon the presence of the HIV-1 specific TATAA sequence. Substituting other eucaryotic "TATA" or "non-TATA" initiators for the HIV-1 TATAA only minimally affected basal expression but dramatically altered Tat-inducibility. These findings suggest a specific combination of functional DNA elements (TATAA and enhancers) is required for optimal Tat trans-activation of the HIV-1 LTR. (Berkhout and Jeang)

A cellular TAR RNA-binding protein. Tat transactivation of HIV-1 gene expression requires viral RNA sequences (TAR) located in the R region of the LTR. Existing evidence suggests that Tat cooperates with cellular factors that bind to TAR RNA during transactivation. A cDNA was isolated from HeLa cells that encodes a TAR RNA-binding protein (TRBP). TRBP activated the HIV-1 LTR and was synergistic with Tat function. TRBP shares a limited homology in a 50 amino acid stretch with *E. coli* ribonuclease III. The remainder of the TRBP amino acid sequence has no discernable relatedness to proteins in existing databases. TRBP is expressed in all tissues examined. (Gatignol and Jeang)

OTHER RETROVIRUSES

Mutational analysis of HTLV-I Tax and the Tax-responsive LTR targets. Ongoing studies examining structure/function relationships of the HTLV-I Tax protein have generated more than 50 point mutants; one set of these mutations permitted the identification of a zinc-binding epitope within Tax. Zinc-binding by Tax is apparently critical for the stable folding of the protein. Other experiments have revealed that stably expressed Tax protein transactivates the HTLV-I LTR through AP-1 motifs. (Semmes and Jeang)

Potential receptor function of the mouse homolog of the gibbon ape leukemia virus receptor. The mouse homologue of the human gene for the gibbon ape leukemia virus (GALV) receptor (Glv-1) was identified using a DNA segment amplified from human DNA by PCR. This

sequence was mapped to mouse chromosome 2 by Southern blot analysis of somatic cell hybrids and positioned on this chromosome using an interspecies genetic cross. Mouse chromosome 2 also encodes a receptor (Rec-2) for the mouse-tropic wild mouse virus M813. Two methods are being used to investigate whether Glvr-1 and Rec-2 could be allelic variants of the same gene. First, Southern blot hybridization with GALV-derived *env* and *pol-env* probes was conducted to

ascertain whether any polynucleotide sequence homology existed between the *env* genes of GALV and M813. While no homology has been detected, all mouse species tested were found to carry numerous copies of GALV-related sequences. Second, human cells transfected with the mouse Glvr-1 gene are being tested for susceptibility to M813.

(Kozak, Adamson, Silver)

Cellular genes involved in MuLV-induced neoplastic disease. Murine leukemia viruses (MuLVs) are capable of transforming mouse cells by mechanisms which include transduction of cellular oncogenes and activation of oncogenes by insertional mutagenesis. Three newly described common viral integration regions involved in tumor induction by three different viruses have now been characterized. 1) The MMTV integration site associated with induction of precancerous hyperplastic nodules was mapped to Chr 9 and represents a novel gene involved in disease induction. 2) The site associated with CasBrE MuLV induction of non-T, non-B cell lymphomas is identical to Fli-1, a site rearranged in Friend virus induced erythroleukemias. 3) A site termed Sfpi-1, also described by others and which is rearranged by Friend SFFV in erythroleukemias, has been independently identified.

(Kozak)

The mouse Friend virus resistance locus, Fv-2. The leukemogenic membrane glycoprotein (gp55), encoded by Friend SFFV, has been reported to bind to the erythropoietin (Epo) receptor and stimulate erythroblastosis. Mice homozygous for the recessive Fv-2 resistance allele are resistant to Friend viral erythroleukemias and Epo induced erythroblastosis. To determine whether Fv-2 encodes the Epo receptor, the Ep_{or} locus was mapped, but its location at the centromeric end of Chr 9 indicates the two genes are distinct. In another series of experiments on Friend virus induced disease, several common integration sites were mapped, one of which was positioned in the region of Chr 9 carrying Fv-2. To establish the proximity of this site with Fv-2, a second cross was generated which could be typed directly for Fv-2. Results indicate that the integration site is at or very near to Fv-2, and experiments are in progress to characterize this site further.

(Chakroborti and Kozak)

Genetic linkage studies in the mouse. One of the major goals of the effort to characterize the mammalian genome is the production of a high density mouse linkage map. A variety of mouse genes have been positioned in the linkage map using somatic cell hybrids and interspecies and intersubspecies genetic crosses. The progeny of these crosses have now been typed for over 200 loci on 20 linkage groups. Some of the genes mapped most recently include the polymerase B gene, a gene causing a defect in spermatogenesis, and a variety of genes expressed in specific cells or tissues. The map locations for several of these genes suggest they may be candidates

for various mouse mutations. Thus, two genes specifically expressed in cerebellum map at or near the neurological mutations, *nervous* and *weaver*, the vitamin K receptor maps to the same chromosome as the gene for warfarin resistance, and a brain cDNA, which appears to encode a transmembrane protein, maps at or near the grey tremor locus. These genes are being characterized in the mutant mice.

(Kozak and Adamson)

The mouse Fv-1 locus. The mouse Fv-1 restriction locus is responsible for the *in vivo* and *in vitro* restriction of subtypes of mouse-tropic murine leukemia viruses. Fv-1n mice restrict B type viruses, and Fv-1b mice restrict N type viruses. N and B type viruses differ in two adjacent amino acid residues in the p30 Gag protein. Site specific mutagenesis has been used to generate viral genomes which differ in only one of these two sites, and we are attempting to generate new viral phenotypes by introducing other substitutions at this site. A virus representing a third subgroup restricted in Fv-1nr has now been cloned; the p30 *gag* gene is currently being sequenced.

(Chakraborti and Kozak).

The integrase genes of leukemogenic and non-leukemogenic murine retroviruses are structurally and functionally distinct. Nucleotide sequences of the IN genes of leukemogenic MCF13 murine leukemia virus (MuLV) and non-leukemogenic MCFM111A MuLV have been compared: 21 amino acids were substituted of which 4 affected hydropathicity; a 4 amino acid insert was also present in MCF13 but not in MCFM111A MuLV. Integrase activities were measured in *in vitro* biological assays and indicated a two-fold greater activity in MCF13 than in MCFM111A MuLV.

(Khan and Kedar)

SIV from stump-tailed macaques is an ancient member of the SIV/HIV-2 group of lentiviruses. Nucleotide sequences of the *gag* and *env* regions of SIV_{stm} were determined. Minimum-length evolutionary trees were constructed based upon nucleotide variation in these regions; a similar branching order of divergence was observed. SIV_{stm} was quite divergent (16% - 17% different) from Asian rhesus and pig-tailed macaque isolates and from SIV isolated from the African sooty mangabey. This analysis indicated that SIV_{stm} is the oldest member of the SIV/HIV-2 group and is the SIV most closely related to HIV-2 isolates.

(Khan and Galvin)

Secondary effect of a mutation which suppresses only RNase H activity in vitro. Specific point mutations in the RNase H domain of the Moloney MuLV and HIV-1 *pol* genes were shown to dramatically inhibit RNase H activity with little or no effect on reverse transcription in *in vitro* assays. Both mutant viruses were replication-incompetent. PCR amplification of transfected cells

demonstrated that minus strand DNA synthesis was aborted before any *env* sequences were reverse transcribed. These results suggest that in addition to the defect noted *in vitro* affecting RNase H, this mutation may also impair the initiation of polymerization of second-strand synthesis.

(Repaske)

MYCOPLASMAS

Serologic Assays of Mycoplasma Antibody in HIV Infections. Mycoplasma metabolism inhibition tests have been carried out on 172 human sera, representing 52 sera from patients with AIDS, 80 sera from HIV seropositive asymptomatic individuals, and 40 sera from HIV seronegative patients. Four mycoplasma antigens were employed in these tests, including *M. genitalium*, *M. pirum*, *M. fermentans*, and *M. pneumoniae*. MI titers in patients in the asymptomatic group to the four mycoplasma antigens did not differ significantly from the control group of patients. However, there was a significant increase in the number of AIDS patients that had high antibody titers (1:128 to > 1:4096) to *M. genitalium* (19.2%) than those in the asymptomatic group (6.3%) or in the control groups (5.0%). Confirmation of these findings in a larger number of AIDS sera is in progress.

(Tully)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00027-24 LMM
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Basic Studies of Mycoplasmas		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Joseph G. Tully Head, Mycoplasma Section LMM, NIAID Others: David L. Rose Research Microbiologist LMM, NIAID		
COOPERATING UNITS (if any) J.B. Baseman, Univ. Texas, San Antonio, TX; J.M. Bove, Univ. Bordeaux, France; S.C. Lo, AFIP, Washington, DC; J. Shin, Dept. Transfusion Medicine, NIH Clinical Center		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Mycoplasma Section		
INSTITUTE AND LOCATION NIAID, NIH, Frederick, MD 21702		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> Mycoplasmas associated with or isolated from tissues of AIDS and non-AIDS patients have been compared biologically and serologically to a collection of reference and recently isolated strains of human origin maintained in this laboratory. Strains of <i>Mycoplasma fermentans</i> and <i>M. pirum</i> from AIDS patients were serologically similar to other reference strains of these species, and for the most part, were similar in biological characteristics. However, some <i>M. fermentans</i> strains were strongly hemolytic to human erythrocytes and some <i>M. pirum</i> strains, associated with human tumor cell lines, exhibited more active attachment features than AIDS-associated strains. All <i>M. prium</i> strains isolated to date possess an organized terminal tip structure, but most strains examined here did not hemadsorb nor express cytopathogenic effects on tissue cells. Five <i>M. genitalium</i> strains were all active in hemadsorption and attachment to glass and plastic surfaces. The potent cytheadherence and cytopathogenic activity of these strains for selected lymphocytic and fibroblastic cell lines correlates well with their attachment properties and the presence of the specific adhesion protein found at the tip structure of these organisms. Mycoplasma serological testing of various human sera from patients with AIDS, from HIV-positive (AIDS-negative) patients, and from control patients were carried out with four <i>Mycoplasma</i> species (including the three species mentioned above and <i>M. pneumoniae</i>). A significant increase in high serum titers (above 1:128) to <i>M. genitalium</i> was found in AIDS patients (19.2%), over similar high titers in HIV-positive (6.3%) or control groups (5.0%). The relevance of this finding is uncertain at present and further serologic studies are in progress with these mycoplasmas. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00190-13 LMM
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Molecular Genetics of Eukaryotic Cells and Their Viruses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. A. Martin	Chief LMM, NIAID
Others:	E. Vicenzi	Visiting Associate LMM, NIAID
	Peter Dickie	Visiting Associate LMM, NIAID
	Hironori Sato	Visiting Associate LMM, NIAID
	Damian Purcell	Guest Researcher LMM, NIAID
	Elizabeth Ross	Biologist LMM, NIAID
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 7	PROFESSIONAL: 6	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>Because of the importance of retroviral long terminal repeats (LTRs) as determinants of tropism and pathogenic potential, <i>in vitro</i> mutagenesis techniques has been used to alter these elements in the context of an infectious molecular clones of the human immunodeficiency virus (HIV). Starting with a replication-incompetent molecular clone of HIV lacking the two NFκB and three Sp1 binding sites present in wild-type LTRs, proviruses containing reconstructed LTRs with individual or combinations of NFκB and Sp1 elements were generated and evaluated for their capacity to produce virus progeny following transfection-cocultivation. Virus stocks obtained exhibited a continuum of replicative capacities in different human T-cell types depending on which element(s) was present in the LTR. Of particular interest was the emergence of second-site revertants containing changes affecting the TATA box.</p> <p>An efficient system to monitor the cell-to-cell spread of HIV was developed employing chronically infected H9 donor cells. Under appropriate conditions of co-cultivation, the synthesis of unintegrated viral DNA, monitored by Southern blot hybridization, occurred between 2 and 4 hrs. following infection; viral proteins were detected 8 to 12 hrs following co-cultivation and progeny virions were released into the medium by 16 hrs. The use of metabolic inhibitors and/or specific antibodies revealed that the cell-to-cell spread of HIV required: 1) the interaction of gp120 with the CD4 receptor and 2) reverse transcription. Light and electron microscopy indicated that cell-cell fusion, occurring within 15 minutes of co-cultivation, mediated the rapid spread of the infection. Unexpectedly, the fusion observed did not appear to involve budding or free virus particles.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00218-10 LMM
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Chemical Studies on Retroviral DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
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Others:	Malcolm A. Martin	Chief LMM, NIAID
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	Elisa Vicenzi	Visiting Associate LMM, NIAID
COOPERATING UNITS (if any) Janet Hartley, Research Microbiologist, LVD, NIAID Paul Torrence, Chemist, LC, NIDDKD		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>The presence of HIV-1 is determined by the presence of P24 viral antigen in an antigen capture assay or by detection of reverse transcriptase (RT) activity. The P24 assay, however, is about 200 fold more sensitive than the RT assay. Various laboratories have reported variable results in attempts to correlate P24 and RT activities. We have qualitatively and quantitatively analyzed the reaction requirements for RT activity and have increased the reaction rate (sensitivity) 500 fold. We find the RT activity now is directly proportional to enzyme (virus) concentration, reaction time and ³²P TTP concentration. With the improved assay, we find a constant relationship between RT and P24 values.</p> <p>A cloned transformed cell line was established which produced defective Moloney murine leukemia virus containing a mutation of a single codon in the RNase H domain of reverse transcriptase. <i>In vitro</i> RT activity is unaffected by this mutation, whereas RNase H activity is 100 fold suppressed. Defective virus were used to infect NIH 3T3 cells, and minus strand DNA synthesis was determined by PCR amplification. After conditions were established to quantitate PCR results, we found initiation of viral DNA transcription to be a rare event: with cells infected with wild type M-MuLV, minus strand strong stop DNA was found within 15 min. These results suggest the mutation affects some prerequisite condition for initiation of RT <i>in vivo</i> but not <i>in vitro</i> with in homopolymer template-primers.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00300-10 LMM

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Aspects of Viral Oncogenesis in Wild Mouse Species and Laboratory Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. A. Kozak Res. Microbiologist LMM, NIAID

Others: A. Chakraborti Visiting Associate LMM, NIAID
J. Silver Medical Officer LMM, NIAID

COOPERATING UNITS (if any)

D. Kabat, Oregon Health Sciences University, Portland; F. Lilly, Albert Einstein College of Medicine, New York; V. Morris, University of Western Ontario, London; E. Rassart, University of Quebec, Montreal; R. Friedrich, Inst. for Med. Virol., West Germany

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Numerous mouse genes involved in virus-induced neoplastic disease have been identified in genetic studies. These genes include endogenous retroviral sequences as well as mouse cellular genes which facilitate or inhibit virus replication. We used the human gene for the gibbon ape leukemia virus (GALV) receptor to identify homologous sequences in the mouse, and we also identified multiple copies of GALV-related sequences in the mouse genome suggesting that this gene may have functioned as a receptor in the evolution of *Mus*. Our genetic mapping of this sequence also suggested possible identity with *Rec-2*, the Chr 2 encoded receptor for a wild mouse retrovirus. In other experiments we characterized three common viral integration regions associated with tumor induction, demonstrating that one represents a novel gene, and one represents a site previously shown to be rearranged following integration by a different virus and resulting in a different disease type. We also demonstrated that the *Fv-2* resistance locus does not encode the erythropoietin receptor, but identified a genomic sequence which maps at or near this resistance gene. Finally, we have used site specific mutagenesis to alter the site in the p30 *gag* region of the AKV MuLV which is thought to interact with the gene product of the *Fv-1* resistance gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00301-10 LMM
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Mapping of Mouse Chromosomal Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: C.A. Kozak Res. Microbiologist LMM, NIAID Others: A. Chakraborti Visiting Fellow LMM, NIAID C. Adamson Chemist LMM, NIAID L. Kohn Section Head LBM, NIDDK F. Gonzalez Section Head LMC, NCI D. Goldman Section Head LCS, NIAAA		
COOPERATING UNITS (if any) M.J. Crumpton, ICRF, London; A. Matus, F. Miescher Inst., Basel; D. Farber, UCLA Sch. of Med.; G. MacGregar, Baylor, Houston; N. Hecht, Tufts Univ., MA; N. Shaper, Johns Hopkins; S. Pestka, UMDNJ, NJ; J. Sikela, Univ. Coll. Hlt. Sci. Center, Denver; A. Beaudet, Baylor, Houston; C. Milstein, MRC, Cambridge; S. Desiderio, Johns Hopkins; D. Joseph, Univ. N. Carolina, NC; N. Moschonas, Univ. Crete, Greece		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) The production of a high density mouse linkage map is one of the major goals of the effort to characterize the mammalian genome. These mapping studies have received increased impetus from the Human Genome Initiative which considers the mouse to be an important model system. Our mapping efforts until recently have been restricted to the analysis of Chinese hamster x mouse somatic cell hybrids by Southern blotting to assign newly identified genes to specific chromosomes. We have now expanded these studies to include analysis of two genetic crosses, an interspecies and an intersubspecies backcross. DNAs from the progeny of these crosses have been typed for a variety of polymorphic reference loci to permit mapping of unknown markers to specific positions on the linkage map. These studies have resulted in the chromosomal mapping of a large number of genes including the polymerase B gene, a gene involved in spermatogenesis, several protein kinase genes, various enzymes, and genes of unknown function with specific patterns of tissue expression. Several of these latter genes map at or near known mouse mutations and therefore are potential candidates for these mutations. Thus, two genes specifically expressed in cerebellum map at or near the neurological mutations nervous and weaver, the vitamin K receptor maps to the same chromosome as the gene for warfarin resistance, and a brain cDNA which appears to encode a transmembrane protein maps at or near the grey tremor locus. These genes and their expression are now being characterized in the mutant mice.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00353-09 LMM

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural and Functional Studies of Mammalian Endogenous Retroviral Sequences

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. S. Khan Microbiologist LMM, NIAID

Others: P. Kedar Visiting Associate LMM, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The integrase genes of leukemogenic MCF13 and non-leukemogenic MCFM111A murine retroviruses were compared in their structure and biological activities. Nucleotide sequence analysis indicated that the two integrase genes were distinct: there were 21 substituted amino acids between MCF13 and MCFM111A, of which 4 affected the hydropathicity profile; in addition, 4 amino acids were present in MCF13 murine leukemia virus (MuLV) which were absent in MCFM111A MuLV. To determine whether the structural differences seen between the integrase genes of leukemogenic versus non-leukemogenic murine viruses affected integrase function, we analyzed the integrase protein activities using an *in vitro* biological system. This assay measured the incorporation of linear viral DNAs, present in cytoplasmic extracts from infected cells, into phi-X174 target DNA. The results indicated that integration by leukemogenic MCF13 was two-fold greater than by non-leukemogenic MCFM111A.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00304-10 LMM
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Pathogenesis of Retroviral Diseases		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> PI: Dr. J. Silver Medical Officer LMM, NIAID Others: Dr. K. Fujita Visiting Associate LMM, NIAID Dr. O. Nahor Visiting Associate LMM, NIAID		
COOPERATING UNITS <i>(if any)</i> Dr. K. Peden, Biochemical Virology Section, LMM		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Viral Biology		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 3	PROFESSIONAL: 3	OTHER: 3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided)</i> <p> The envelope gene of several retroviruses, including HIV, appears to play an important role in pathogenesis. Dr. Orit Nahor tried to construct a non-infectious derivative of HIV that would express envelope constitutively or conditionally in stably transfected cells. Starting with <i>gag-pol</i> deleted HIV clone that expressed envelope in transient transfection assays, Dr. Nahor substituted the hygromycin resistance marker for part of the <i>nef</i> gene. For reasons we do not yet understand, this interfered with envelope expression. Additional constructs are being pursued with the goal of investigating whether envelope expression, per se, is cytopathic, and whether cytopathicity requires co-expression of CD4. Dr. Kazunobu Fujita, working in collaboration with Dr. Keith Peden, investigated molecular changes in HIV associated with adaptation to growth in tissue culture cell lines. Dr. Peden had developed and characterized an infectious molecular clone of HIV that showed adaptation to growth in H9 and U937 cells after forced passage in H9 cells. Dr. Fujita used PCR and a screening technique sensitive to single base changes in bulk amplified DNA, to identify two regions in the envelope gene that differed between the adapted and parental viruses. Dr. Peden showed that these changes were sufficient for enhanced growth in H9 and U937 cells. The location of the changes suggests that alteration in binding of HIV to CD4 and propensity to fuse after binding are responsible for enhanced growth in certain cell lines. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00415-07 LMM

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. A. Martin	Chief	LMM, NIAID
Others:	K. Strebel	Senior Staff Fellow	LMM, NIAID
	F. Maldarelli	Staff Fellow	LMM, NIAID
	L-J Chang	Visiting Associate	LMM, NIAID
	K. Peden	Guest Researcher	LMM, NIAID
	R. Willey	Biologist	LMM, NIAID
	P. Mounts	IPA	LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

7

PROFESSIONAL:

5

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Intracellular transport and processing of the HIV-1 envelope precursor glycoprotein gp160 proceeds via the endoplasmic reticulum (ER) and Golgi complex and involves proteolytic processing of gp160 into the mature virion components, gp120 and gp41. We found that co-expression of gp160 and human CD4 in HeLa cells severely impaired gp120 production due to the formation of intracellular gp160/CD4 complexes. This CD4-mediated inhibition of gp160 processing was alleviated by co-expression of the HIV-1 encoded Vpu protein. Vpu is a 16 kD phosphorylated membrane protein which has previously been shown to enhance the release of viral proteins and progeny virions from infected cells. It has recently been reported that a single bicistronic mRNA is translated into Vpu and the HIV envelope glycoproteins. The co-expression of Vpu and CD4 in the presence or absence of gp160 resulted in increased degradation of CD4. Although the mechanism(s) responsible for the Vpu effect is presently unclear, our results suggest that Vpu may destabilize intracellular gp160/CD4 complexes without directly interacting with either protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00437-07 LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Biology and Genetics of the AIDS Retrovirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. D. Hoggan	Senior Scientist	LMM, NIAID
Others:	G. Englund	Biologist	LMM, NIAID
	M. A. Martin	Lab Chief	LMM, NIAID
	C. R. Wood	Staff Scientist	MCG, Genetics Inst.
	P. Szklut	Biologist	MCG, Genetics Inst.

COOPERATING UNITS (if any)

Molecular and Cellular Genetics, Genetics Institute, Cambridge MA.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00438-06 LMM

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Biology of Cellular Oncogenes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. Santos Visiting Scientist LMM, NIAID

Others: T. Bryan Microbiologist LMM, NIAID
A. R. Nebreda Visiting Fellow LMM, NIAID
M. Benito Guest Researcher LMM, NIAID
F. Segade Guest Researcher LMM, NIAID
S. Venkatesan LMM, NIAID

COOPERATING UNITS (if any)

George VandeWoude, ABL, BRI, NCI-FCRDC
Paul Wingfield, Office of the Director, NIH

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Terminated

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00467-06 LMM

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV-I Particle formation, RNA packaging and Virus Morphogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	S. Venkatesan	Medical Officer	LMM, NIAID
Others:	Elizabeth Ross	Microbiologist	LMM, NIAID
	M. A. Martin	Chief	LMM, NIAID

COOPERATING UNITS (if any)

Thomas Fuerst, Staff Scientist, Med-Immune Corp., Gaith., MD; Jan Orenstein, Pathologist, GWU School of Medicine, Washington, DC

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)

Recombinant vaccinia viruses expressing the HIV-I protease, p55 GAG, p41 GAG, complete GAG-POL and truncated GAG-POL precursor proteins were constructed. Protein expression was confirmed by immunological procedures. Using the above recombinants, we demonstrated that self-assembly of p55 GAG protein is sufficient to form the framework of the nascent human immunodeficiency virus (HIV-1) particle. The particles which budded from the cells infected with a vaccinia-GAG construct were mostly spheres with a concentric ring of electron dense material. Expression of the GAG and POL proteins resulted in mature particles containing a condensed core which assumed the nucleoid structure characteristic of lentiviruses. When the POL frame in the GAG-POL ORF was truncated at the end of the protease domain, p55 GAG processing was markedly reduced and the maturation of the resultant particles was defective. We have extended these findings to show that HIV-I protease expressed from the vaccinia vector can process exogenously substrates such as denatured GAG protein expressed in E. coli or synthetic peptide substrates. However, this protease failed to process particle associated GAG protein whether in the form of purified HIV particles or GAG particles expressed from the vaccinia recombinant.

We have expressed protein cleavage mutants of GAG using the vaccinia vector. Mutations that eliminate the myristoylation or the nucleic acid binding CYS containing motif (NBCys) of the p9 subunit of GAG protein have been introduced into GAG-POL expression vector to examine whether they interfere with capsid formation and RNA packaging. We have developed a simple in vivo model for HIV-I RNA packaging. A subgenomic HIV RNA encompassing R, U5 and U3 elements surrounding the minimal packaging sequence was expressed from the T7 promoter using vaccinia T7 polymerase vector. Co-infection with GAG or GAG-POL recombinant vaccinia viruses lead to HIV particles that selectively packaged the mini HIV RNA. This system is being used to define the molecular parameters of RNA packaging. It is our eventual goal to develop molecular strategies to interfere with RNA packaging and capsidation. Finally, to understand the biochemical processes of HIV provirus synthesis and its subsequent integration, we have over-expressed and partially purified the HIV-I integrase and the RNase H associated with the 15 kDa C-terminal cleavage product of the p64 RT.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00527-04 LMM	
PERIOD COVERED October 1, 1990 to September 30, 1991			
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Basic Studies of Mycoplasmas			
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)			
PI: T.S. Theodore		Research Microbiologist LMM, NIAID	
Others: M.A. Martin		Laboratory Chief LMM, NIAID	
K.A. Clouse		Senior Staff Fellow CBER, NIAID	
G. Englund		Biologist LMM, NIAID	
COOPERATING UNITS (if any)			
LAB/BRANCH Laboratory of Molecular Microbiology			
SECTION Mycoplasma Section			
INSTITUTE AND LOCATION NIAID, NIH, Frederick, MD 21702			
TOTAL MAN-YEARS: 2		PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews			
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)			
<p>Heterogeneity among AIDS retroviral genomes is a distinct feature of these Lentiviruses. Molecular cloning, restriction enzyme analysis, nucleotide sequencing, and polymerase chain reaction techniques were used to characterize isolates of Human Immunodeficiency Virus (HIV). The objective was to obtain information pertaining to the structure and diversity of HIV with respect to its pathogenicity and antigenic variability. In contrast to the numerous T cell lymphotropic HIV isolates we have cloned, experiments are in progress to obtain molecular clones from macrophagotropic isolates. It has been suggested that macrophages are the primary reservoir of HIV, sustaining a persistent infection in individuals for many year.</p> <p>A survivor T cell line (ACH-2) constitutively produces low levels of HIV particles. Compared to the infectious clone (pNL4-3) and pLAVbru, which produced viral particles after 7 days, ACH-2 viral supernatants produced two to four logs (TCID-50) less virus after 15 days. This cell line may provide information on the pathogenicity of HIV as it relates to persistence and/or latency and viral induction.</p>			

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00528-04 LMM
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional studies of HIV-1 regulatory Proteins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S. Venkatesan	Medical Officer LMM, NIAID
Others:	S. M. Holland H. S. Park	Commissioned Officer Fogarty Fellow LMM, NIAID LMM, NIAID
COOPERATING UNITS (if any) Eugenio Santos, Visiting Assoc. LMM, NIAID; Paul Wingfield, Chief, Prot. Exp. Lab. OD, NIH; Peter Dickie, Visiting Associate, LMM, NIAID.		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION Biochemical Virology Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">3</div>	PROFESSIONAL: <div style="text-align: center;">3</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>The replication of of human immunodeficiency virus (HIV) is dependent on the function of two small virus coded regulatory proteins, TAT and REV. REV protein acts post-transcriptionally to modulate the splicing, extra-nuclear transport and/or translational utilization of unspliced or partially spliced viral RNAs. REV mediates its function by interacting with a highly structured viral mRNA sequence, RRE (REV responsive element) of about 236 nt. located in the ENV ORF. A) Studies using 80 or so RRE mutants have demonstrated that i) REV binding to RRE RNA is necessary but not sufficient for REV function in vivo; ii) REV binding to RRE is dependent on the presence of a 5'(50)CACUAUGGG(58)3' in the context of an unique secondary structure; iii) of the 56..GGG..58 sequence the G-56 is critical and a minimum of 2 Gs are required for RRE function; iv) other homopolymers cannot substitute; and v) mutations distal to the 3 Gs that result in base-pairing of the Gs eliminate binding. B) We have identified a putative nuclear factor that binds to a sub-domain of RRE <i>in vitro</i> distinct from the REV recognition region. C) We have devised a chimeric RRE construct replacing the REV responsive domain with an unrelated RNA, namely the MS2 phage translational operator sequence. This chimera was activated by a REV-MS2 coat protein fusion that tethered REV to the heterologous RNA via the MS2 protein. These fusion proteins have allowed us to dissect the function of distinct domains within REV and RRE. The above molecular studies have enabled us to devise genetic therapeutic approaches using RNA decoys to impede REV function in natural HIV infection.</p> <p>We have extended and refined our earlier studies on the effect of HIV-1 NEF protein on LTR transcription. Stable Hela cell and Jurkat lymphoid cell lines expressing NEF from HIV-1 LTR, CMV early promoter or MMTV or RSV LTR have been developed. NEF expression in these cell lines was consistently associated with repression of HIV-1 LTR transcription. Although the mechanism(s) of NEF induced repression were not deduced, they are unlikely to be mediated by GTP binding or GTPase activities. NEF from four different HIV-1 isolates were expressed and purified. All the NEF proteins were devoid of GTP binding or GTPase activities although they possessed measurable auto-kinase activity.</p> <p>To examine pathogenic potential of NEF, several transgenic mouse lines expressing NEF either from the HIV-1 LTR or MMTV LTR were constructed. HIV-1 LTR linked NEF expression was confined to skin, particularly in the basal cells of the epithelium and the Langhans cells. Interestingly, NEF expression was associated with papillomatous skin lesions that varied in severity depending on the level of NEF expression. MMTV LTR driven NEF expression was observed in the mammary and salivary glands, seminal vesicles and the testes. In all these organs, no obvious pathology was associated with NEF expression. A small number of MMTV NEF transgenic mice also expressed NEF in the skin where it was associated with papillomas.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00546-03 LMM
PERIOD COVERED October 1, 1989 to September 30, 1990		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Regulation of Human Retrovirus Gene Expression		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> PI: A. B. Rabson, M.D. Others: T. J. Seidner Guest Researcher LMM, NIAID W. Turner Biologist LMM, NIAID		
COOPERATING UNITS <i>(if any)</i> Malcolm A. Martin, M.D., LMM, NIAID; Elizabeth Ross; Alicia Buckler-White, Ph.D., Georgetown University.		
LAB/BRANCH Laboratory of Molecular Microbiology		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> Terminated		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00547-03 LMM

PERIOD COVERED

October 1, 1989 to September 31, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanism(s) of Human Retrovirus *trans*-Regulatory Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K-T. Jeang Medical Officer, Research LMM, NIAID

Others: A. Gatignol Visiting Fellow LMM, NIAID
B. Dropulic Visiting Fellow LMM, NIAID
Y. Chang Staff Fellow LMM, NIAID
O. J. Semmes, IV Adjunct Scientist LMM, NIAID
M. Martin Chief LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

5

PROFESSIONAL:

5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to study the mechanism by which HIV-1 Tat and HTLV-I Tax proteins activate transcription. Relevant to Tat function are the following findings: 1) Intracellularly, Tat functions primarily at the level of initiation of transcription. 2) The action of Tat does not require a "short RNA" intermediate. 3) Tat transactivation of the HIV-1 LTR requires DNA elements that includes both enhancer and TATA sequences. 4) For full function, Tat interacts with cooperating cellular factors. One such cellular factor has recently been cloned in this laboratory.

For HTLV-I Tax we have made the following observations: 1) Tax is a zinc-binding protein. Zinc is required for the stable folding of the protein, and the epitope for zinc-binding within Tax has been elucidated. 2) The Tax-responsive sites within the HTLV-I LTR are functionally AP-1 sites.

Two emerging areas of interests in the laboratory involve studies on ribozymes and on the *rel* protein family. In this regard we have developed specific catalytic RNAs targeted to the HIV-1 genome. We have also been studying the role of *v-rel* and *c-rel* in the regulation of the HIV-1 and HTLV-1 LTRs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00588-02 LMM

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Simian Immunodeficiency Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. S. Khan Microbiologist LMM, NIAID

Others: T. Galvin Guest Researcher LMM, NIAID
L. Lowenstine Assoc. Professor Univ. of
Calif. Davis

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

.6

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

SIV_{stm} was isolated from a rhesus macaque which had developed an AIDS-like disease upon inoculation with stored lymph node tissue of an Asian stump-tailed macaque; the latter monkey had died in 1977 during an epidemic of acquired immunodeficiency and lymphoma at the California Regional Primate Research Center. Nucleotide sequence analysis of PCR-amplified fragments of the LTR and gag regions indicated that SIV_{stm} was a novel member of the SIV/HIV-2 group. Nucleotide sequences of other regions of SIV_{stm} were also determined. Minimum length evolutionary trees were constructed based upon nucleotide variations in the gag and env regions. A similar branching order of divergence was seen in both cases. Previously known SIVs from sooty mangabey and macaques were only 11% - 13% different from one another and 24% - 30% different from HIV-2s. On the other hand, SIV_{sm} was quite divergent from the other SIVs (16% - 17% different) and only 21% - 25% different from the HIV-2s. The data indicated that SIV_{stm} is the oldest SIV of the SIV/HIV-2 group, reported to date and the most closely related SIV to the HIV-2s.

LABORATORY OF PARASITIC DISEASES
1991 Annual Report
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Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Disease
Summary - October 1, 1990 - September 30, 1991

ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

As one of the largest Intramural NIAID laboratories there is a great deal of coming and going of professional personnel over the course of a year. The following is a fairly complete account of the turnover, with a listing first of those who left during the past fiscal year.

From the Malaria Section Dr. Isabella Quakyi, who came to LPD from Ghana, and has played an important role with the gametocyte work, joined a malaria research group at Georgetown University. Dr. Andrew Waters, a molecular biologist from the U.K. who had been with Dr. McCutchan, took a position with a research institute in Holland. Dr. John Adams, a senior Staff Fellow with Dr. Miller, went to Notre Dame University as an Assistant Professor. Dr. Antoniana Krettli, who had been on a sabbatical from her position with Fio-Cruz in Belo Horizonte, Brazil, to work with us on immunology of murine malaria, returned to Brazil. Three more people from the Malaria Section finished their Visiting Fellowships: Dr. Marcel Huber, who was with the entomology group, will return to Switzerland; Dr. Xiandong Fang decided to take another postdoctoral fellowship at Rockefeller University before returning to China; and Dr. Annie Jonah-Walker, originally from Ghana via a Ph.D. in Germany, is taking a position locally with the Biomedical Research Institute. From the Immunology and Cell Biology Section we lost a key person in Dr. Edward Pearce who moved on to an Associate Professorship at the Ithaca campus of Cornell University. We will also miss Dr. Paul Brindley, an LPD favorite, who went back to his native Australia to a key position at an agricultural research institute. Dr. Tom Brodin returned to Sweden where his future position is still under negotiation. Dr. David Mallinson went back to Scotland where he has an academic position. Dr. Chris King, who came originally to LCI is a Medical Staff Fellow and then to LPD for a very productive three years in the Clinical Parasitology Section, took a faculty position at Case Western Reserve University. From the same Section, Dr. Amy Klion secured an Infectious Disease Fellowship at the University of Iowa so she could be with her husband, who started a Neurosurgery residency. Dr. Edgar Lobos was a third departure from Clinical Parasitology, deciding to take a job in Switzerland where he received his Ph.D., instead of taking a position that had been offered in Guatemala. There were two departures from the Host-Parasite Relations Section: Dr. Yuhui Xu, a pathologist from China, stayed only two years of his Visiting Fellowship in order to take another post-doctoral position in Boston at the Dana Farber Institute to learn special techniques; Dr. Bach-Yen Nguyen finished her Medical Staff Fellowship to take a position with the Infectious Disease group of NCI. Finally, from the Section of Biochemistry and Physiology, Dr. Juan Carlos Engel, a Visiting Associate from Argentina, left to take a position at the University of California at San Francisco.

In addition to the above, there were a number of professionals who came to LPD from

abroad for shorter periods of time to work with specific LPD staff members. Dr. Alok Bhattacharya from India came on the final year of his short-term Rockefeller Foundation fellowship to work in Dr. Diamond's lab on immunobiology of *E. histolytica*. Dr. Fred Gyang from Ghana came on a special fellowship to work with Dr. Tom Wellems in the Malaria Section. Dr. Gerusa Dreyer came from the Recife, Brazil branch of the Fio Cruz Institute as part of a collaborative project being developed by Dr. Ottesen of our Clinical Parasitology Section for work on filariasis.

LPD also had the distinction of hosting three Fogarty Scholars during the past year. Dr. Peter Perlman of Sweden was at NIH for a short time and will return again during the year to interact with members of the Malaria Section. Dr. David Mirelman of the Weizmann Institute in Israel spent seven months in Dr. Diamond's lab working on amebiasis. Dr. Zilton Andrade, a well-known expert in pathology of parasitic diseases and a colleague of Dr. Cheever's from many years back, was with us for several months and will return again for several months.

Dr. Dan Zilberstein of Israel, a former Visiting Fellow with Dr. Dwyer of LPD, is back for about six months as a Visiting Scientist. Dr. Don Krogstad, an Associate Professor at Washington University in St. Louis, came to spend a year with the Malaria Section of LPD under an IPA arrangement.

Three physicians came to LPD as research fellows although most of their time will be spent in the laboratory. Dr. Christopher Plowe came to us as a Medical Staff Fellow from a residency at Cornell and an M.P.H. degree from Columbia University to work in the Malaria Section. Dr. James McCarthy, who trained in Australia and just finished an Infectious Disease Fellowship at University of Maryland, will be working in the Clinical Parasitology Section as a Visiting Associate. Dr. Isam Eltom is a pathologist and recent Ph.D. from the Sudan who has come to work in Dr. Cheever's lab of LPD.

The Malaria Section had the greatest number of new research fellows. Dr. Alon Warburg, an entomologist from Israel, and Dr. Shahabuddin, a recent Ph.D. from Bangladesh via graduate studies in the U.K., are Visiting Fellows who started this year. Dr. Greg Lanzaro, a recent Ph.D. from the West Coast, and Dr. Dennis Bryant, a recent Ph.D. from the midwest, will be working with materials from the *Anopheles* mosquito vector project in Mali, West Africa. They will work in Mali for short periods, but will be based locally, Lanzaro in Dr. McCutchan's lab and Bryant with a microbiology group at the University of Maryland.

The Immunology and Cell Biology Section had two additions: Dr. Megan Williams, a recent Ph.D. immunologist from Harvard, and Dr. Jeff Actor, a Ph.D. molecular biologist from Brandeis University.

With regard to foreign travel, only research activities (TDY) will be reported. First of all, Dr. Richard Sakai went to Bamako, in Mali, West Africa, where he will be the entomologist in residence with the Malaria Section's mosquito vector project. He will be living there for the next few years doing field research on the *Anopheles gambiae* complex of mosquitoes. Dr. Gwadz traveled back and forth to Mali several times in setting up the project which is partially supported by AID. Dr. Gwadz also continues to be associated with the Egypt-Israeli scientific collaborative project which brings him to the Middle East several times a year. Dr. Ottesen traveled world-wide in his association with the WHO-supported trials of ivermectin treatment of filariasis, helping to set up the trials. Drs. Nutman and Klion

spent several weeks in Benin, West Africa, at an endemic site for *Loa loa* infection, studying the immunology of this parasitic infection. Dr. Neva visited Mexico to renew his collaboration with Dr. Velasco on leishmaniasis and recruited a patient from Tabasco State with DCL to come to the NIH Clinical Center. Dr. Andrea Cooper spent a week in Honduras working with Dr. Carlos Ponce and his wife on leishmaniasis.

HONORS AND AWARDS

All of the permanent professional staff, and many of the senior nontenured staff serve on Editorial Boards of journals and frequently review manuscripts for journals. Clearly this activity is evidence of the high standing of LPD scientists by professional peers. Also, many of the Staff serve on Ad Hoc Committees for granting agencies or foundations, and are invited to participate in workshops and scientific meetings. Such activities are not cited individually here unless they deserve special mention.

Dr. Louis Miller received the Distinguished Service Award from the Department of Health and Human Services. He was also elected to membership in the Institute of Medicine.

Dr. Eric Ottesen was awarded the Meritorious Service Medal of the U.S.P.H.S. Commissioned Corps.

Dr. Tom Nutman received the Commendation Medal of the U.S.P.H.S. Commissioned Corps.

RESEARCH PROGRESS

Immunology & Cell Biology Section

CYTOKINE RESPONSES IN EXPERIMENTAL SCHISTOSOMIASIS Down regulation of Th-1 type cytokines probably aids schistosome worm survival. Last year it was reported that *S. mansoni* infected mice, in contrast to immunized mice, had a Type-2 response which is characterized by production of IL-4 and IL-5 (resulting in IgE and eosinophil increases, respectively). Evidence for another cytokine regulated mechanism via IL-10 was found which would tend to prevent the schistosome worm from immune damage. This involves suppression of INF-gamma mediated macrophage activation. When IL-10 is added to macrophage cultures, the ability of INF-gamma to activate macrophages in killing of young schistosomes is suppressed. Moreover, this action of IL-10 in suppressing macrophage activation is greatly enhanced by IL-4 and another cytokine, TGF-B (Pierce, Oswald, James and Sher).

Evidence that suppression of the Th-1 cytokine response induced by experimental schistosome infection is not antigen specific. Since the schistosome-infected mouse exhibits suppression of Th-1 responses, it was of interest to determine whether the responses to other antigens would be similarly affected. When infected mice were immunized with sperm whale myoglobin, the mice exhibited a marked suppression of IL-2 and INF-gamma responses to the myoglobin, whereas IL-4 responses to myoglobin were normal. This suggests that schistosome-infected patients might have difficulty in mounting TH-1 dependent immune responses (Sher and Berzofsky of NCI).

An example of experimental cytokine immunotherapy. By injecting antibodies against IL-2, it was possible to partially block granuloma formation and ablate subsequent fibrosis around *S. mansoni* eggs. Interestingly, this treatment also led to divergent results in two cytokine-mediated responses - (1) a marked reduction in eosinophilia (blood and tissue) but (2) an elevation in serum IgE. Thus, in this model, IL-2 has a potent effect on egg-induced Th-2 responses (Sher, Xu and Cheever).

APPLICATION OF RECOMBINANT SCHISTOSOME ANTIGEN

BCG - paramyosin recombinants. As mentioned in last year's report, a collaborative project with Med Immune, Inc. was initiated to make an experimental vaccine from a recombinant BCG vector that would express paramyosin, a constituent of schistosome worms. The recombinant contains a 1.3Kb fragment of the paramyosin gene and expresses a product about 50% the size of the native molecule. When administered to mice without any other adjuvant intradermally or subcutaneously, the recombinant induced significant paramyosin specific IL-2 and INF-gamma T-cell responses when measured 6 to 8 weeks after inoculation. Interestingly, neither IL-4 or IL-5 T-cell responses or antibodies against paramyosin could be detected in the same animals. These results suggest that BCG vectors may preferentially induce Th-1 T cell responses. Studies testing protective capacity of these experimental recombinant antigens are in progress (Pierce, Oswald, and Sher in collaboration with De la Cruz).

Screening clones of parasite material for epitopes that will react with T-cells from infected or immunized mice. About 900 clones from a lambda Gt11 adult worm cDNA expression library were first screened with polyvalent antischistosome antiserum. These clones, in turn, were expressed and the products screened in 96-well plates against T-cells from vaccinated mice. A number of recombinants were found to consistently and specifically produce significant levels of interferon gamma from the immune T-cells. The clone giving the strongest signal was selected for further study and its 5kb insert is currently being sequenced. In a similar fashion a second battery of clones have been obtained by screening a library with antisera against schistosome egg antigens. These clones are being used to screen T-cells from infected mice to identify antigens relevant to egg-induced immunopathology (Oswald, Williams and Sher).

DEVELOPMENTAL
BIOLOGY AND T-CELL
IMMUNE RESPONSES IN
LEISHMANIASIS

Changes in Promastigote surface LPG correlate with infectivity of sandfly vector. Lipophosphoglycan (LPG), which is the major surface constituent of the leishmanial promastigote, undergoes an increase in oligosaccharide units and a replacement of terminal galactose with arabinose units, as it develops from the noninfective

log phase to stationary infective forms in the sandfly gut. Since the organisms bind to the insect gut wall during growth and later migrate to the mouth parts, the role of LPG in midgut binding was studied directly by incubating dissected *P. papatasi* midguts with $^{125}\text{I}_2$ labelled organisms from log and stationary phases of growth. Metacyclic promastigotes did not bind while log phase organisms bound to the midgut; moreover, binding of log phase organisms could be inhibited by LPG. (Sacks and Brodin).

Antibodies against LPG as a transmission blocking vaccine for leishmania. Since the results above suggested that LPG functions to maintain organisms in the midgut for growth, the effect of antibody in the bloodmeal against LPG was tested for ability to interfere with parasite development and serve as the basis of a transmission blocking vaccine. When female flies were membrane fed on heparinized blood from LPG immunized mice, most flies lost parasites while control flies retained their infections. Results suggest that anti-LPG antibodies persist long enough in the digesting blood meal to react with the surface of newly transformed promastigotes, and that the presence of the anti-LPG antibodies disrupts normal development of midgut promastigotes and promotes loss of infection in the fly. (Sacks and Pimenta).

T-cell responses in human and murine leishmaniasis. Several different approaches are being used to identify important cell-mediated responses indicative of protective immunity. One approach being applied is to screen for fusion proteins in cDNA libraries constructed in a lambda ZAP vector. The first screening was done for clones reacting with serum from a visceral leishmaniasis patient and a second screening of positive clones was done with cells from a muco-cutaneous case using T-cell proliferation and gamma interferon production as indicators. The recombinant antigen was excised from the lambda phage and passed to a transformed *E. coli*. Seven positive clones have again been selected for further characterization. (Karp and Sacks with Donaldson and Wilson from the Univ of Iowa as collaborators.) A second approach has been to use infected autologous monocytes as antigens and measure proliferative responses of T-cells from the same patients. In addition, the T-cell response has been further dissected by depleting CD4, CD8, or gamma delta cells. In general, T-cells from cutaneous and muco-cutaneous patients were unable to proliferate or produce INF-gamma in the absence of CD4 cells. Some of the proliferative responses could be restored by adding IL-2. However, it is clear that induction of a proliferative response is not always accompanied by production of gamma interferon (Sacks, Cooper and Neva).

A final approach that is being developed is to examine and identify cell types in a regional lymph node after leishmanial infection in the BALB/c mouse. In addition, cytokine levels in the lesions, as assayed by PCR reactions, will be followed over a period of time. These studies have just started (Cooper and Sacks).

BIOCHEMISTRY AND
MOLECULAR BIOLOGY
OF LEISHMANIA

Molecular cloning of *Leishmanial* secretory acid phosphatase(Sacp) gene(s). Previous work has demonstrated the events that lead to eventual biosynthesis of Sacp in the Golgi compartment of the organism.

Molecular cloning of the Sacp gene(s) is now underway. Pulse-field gels of genomic DNA from various *Leishmania* species suggest that the gene(s) are present on two different sized chromosomes of species causing visceral disease, but only on one chromosome of species associated with cutaneous disease (Mann, Mallinson and Dwyer).

Identification and purification of enzymes present on surface membrane of *Leishmania*. In addition to the proline and methionine transport proteins reported last year, an adenosine transporter was identified on the membrane of promastigotes. Again, this was made possible by the use of several radiolabeled affinity binding reagents. The proline transporter is being cloned (Mann, Zilberstein and Dwyer). S-adenosyl methionine was shown to act as a methylating agent (i.e., a methyl group donor) and to greatly increase activity of leishmanial 3' nucleotidase (Mann and Dwyer).

Characterization of *L. donovani* amastigotes grown *in vitro*. Previous workers have described culture systems in which leishmanial parasites can be grown freely as amastigotes outside of cells. These systems utilize elevated temperature (37° C) as one requirement, but the organisms grown this way have usually not been well characterized. A clone of *L. donovani* was adapted to growth at 37°C in a high serum content medium by P. Doyle last year and has been studied further. Interestingly, this line was initiated from a clone which appeared to have lost virulence in that it was incapable of producing metacyclic (infective) forms. The line can now be manipulated back and forth to grow as amastigotes at 37° C or as promastigotes at 26°C. The amastigotes are infectious for human monocytes. This line of *leishmania* will be useful for a variety of studies on gene-regulated differentiation and development (Dwyer, Sacks and Zilberstein).

IMMUNE REGULATION
IN EXPERIMENTAL
TOXOPLASMA
INFECTION

More evidence for the importance of CD8+ lymphocytes in experimental toxoplasmosis. One system used to study immune function is to immunize mice with an attenuated (Temperature sensitive) strain of *T. gondii* and then examine events after restimulation (boosting) with antigen. Under these circumstances, CD8+ lymphocytes from vaccinated mice were shown to produce significant amounts of INF-gamma, a cytokine previously shown to be required for protective immunity. But perhaps more importantly, the CD8+ cells were shown to be specifically cytolytic when allowed to react with infected or antigen pulsed bone marrow derived macrophages. Such target cells are used because they express high levels of class I MHC molecules. This system of pulsing target cells is being used to identify the parasite epitope(s) recognized by the cytotoxic lymphocytes (Gazzinelli, Denkers and Sher of LPD and Shearer and Hakim of NCI).

Cell mediated immunity in control of chronic toxoplasma infection. Mice were infected with the ME49 strain of *T. gondii* which establishes a chronic infection in order to examine how

perturbation of the cell-mediated immune system might affect the toxoplasma infection. Treatment of the mice with monoclonal antibodies (Mabs) against CD4+ cells, plus anti CD8+ cells, resulted in fulminating fatal encephalitis. Giving anti INF-gamma Mabs also led to a fatal encephalitis. If anti CD8+ Mabs were given, there still was a fatal outcome but at a slower rate. In contrast, depletion of CD4+ cells with Mabs had no effect on mortality. These results, coupled with the observations described above, indicate that CD8+ lymphocytes are the primary effector cells controlling reactivation of toxoplasma infection. (Gazzinelli and Sher).

Toxoplasmosis in a murine retroviral (MAIDS) model. Reactivation of a murine retrovirus which produces an immunodeficiency state with some similarities to human AIDS (MAIDS) was studied in collaboration with Dr. Morse's group of NIAID. MAIDS infection was found to cause a partial reactivation of latent toxoplasma infection. This was associated with a decrease in INF-gamma response and enhanced Th2 cytokine responses. Depletion of CD8+ cells in MAIDS-infected animals resulted in rapidly fatal encephalitis. These results suggest that loss of INF-gamma and CD8+ functional activity in human AIDS patients may be important factors in development of toxoplasmic encephalitis (Gazzinelli, Sher and Cheever of LPD and H. Morse of LIP).

Clinical Parasitology Section

IMMUNOREGULATION OF B AND T CELL RESPONSES Factors controlling IgG subclass responses in parasitic infections. The regulation of antibody responses in the different IgG subclasses was examined *in vitro* and *in vivo* in parasitic as well as in nonparasitic diseases. In clinical situations associated with hyper IgG states (such as filarial infections or the hyper IgE syndrome), there is marked increase in absolute amounts of both IgE and IgG4. To investigate this response *in vitro* the precursor frequency of B cells producing different IgG subclasses was studied with ELISPOT assays in normals and patients with active helminth infections. These data demonstrated that parasite antigens stimulate increased B cell precursor frequencies to all subclasses and IgE, in contrast to a nonparasite antigen such as tetanus toxoid which stimulated only IgG1, IgG2, and IgG3 responses. Furthermore, IL-4 was essential in the generation of both IgG4 and IgE, but not the other IgG subclasses. In contrast, endogenously produced IFN-gamma had an inhibitory role in these responses but was not isotype specific. The data suggest that INF-gamma acts on IgG4 in the same manner as it does for IgE, suggesting a linkage between these two isotypes (King, Ottesen, Nutman).

Immunoregulation in filarial infection. The cytokine control of eosinophilia as seen in helminth infections was studied at the level of the T cell. IL-5 had previously been shown to be present in lymphocytes of patients with eosinophilia to a greater extent than those without eosinophilia, and this IL-5 was clearly inducible by parasite antigen if the patient had been previously sensitized. In contrast, neither IL-3 nor GM-CSF appear to regulate the reactive eosinophilia seen in parasitic infections. By ELISPOT assays to measure the frequency of T cell-producing cytokines, there was a marked increase (50-200 fold) in IL-5 secreting T cells, in contrast in only one to three fold increases in IFN-gamma and GM-CSF production to parasite antigens.

Furthermore, in two studies of treatment of human filarial infections, the absolute dependence of eosinophilia on IL-5 was demonstrated as IL-5 levels in the serum were consistently shown to precede the rise in peripheral blood eosinophilia (Mahanty and Nutman).

Patients with generalized onchocerciasis fail to mount a lymphocyte proliferative or cytokine response to parasite antigen, whereas exposed individuals in the same area who are presumably "immune" do respond to parasite antigens. In particular, cells from the "immune" patients produce IL-2 and this cytokine then appears to induce IL-5 (Nutman and Mahanty). The nature of the parasite-specific anergy seen in asymptomatic individuals with microfilaremic Bancroftian filariasis has been examined by precursor frequency analysis (i.e., relative numbers of T and B cells capable of a specific response). Results indicate that in this hyporesponsive state there is a decreased frequency of antigen reactive T and B cells. Preliminary evidence suggests that this immune tolerance may be due to active suppression by a sub-population of lymphocytes that produce the cytokines IL-10 and TGF-beta (Nutman, King, Ottesen, Mahanty).

SPECIFIC ANTIGENS INVOLVED IN IMMUNE RESPONSE TO FILARIAL INFECTIONS A recombinant antigen for early diagnosis of onchocerciasis.
A recombinant antigen (OV16) isolated from a cDNA expression library derived from *O. volvulus* adult worms has been shown to be consistently recognized by patients with onchocerciasis. A few molecular tricks were required to achieve expression of the antigen in a fusion product (Maltose-binding protein) that greatly helps to purify the antigen. OV 16 antigen has now been evaluated in a blinded comparative trial coordinated by WHO which found the antigen to be specific and superior to other antigens. Moreover, OV16 was found to detect anti-*onchocerca* antibodies in experimentally infected chimpanzees 3 to 12 months before infections could be detected parasitologically. The WHO is going to develop and use OV16 in further field studies (Lobos, Ottesen and Nutman).

Recombinant "immune" antigen in Bancroftian filariasis. Previous work described in these reports identified a 43kD larval antigen that was recognized by sera from infection free ("immune") individuals in the Cook Islands of the Pacific. This antigen has now been cloned from a *W. bancrofti* expression library and the sequence of the gene has been determined (Raghavan, Nutman and Ottesen).

MOLECULAR DEFINITION OF FILARIAL ANTIGENS Antigens from *W. bancrofti*. The 43 kD "protective" antigen described above was found to have a sequence with significant homology to the enzyme, chitinase. By *in situ* hybridization mRNA for the antigen was found in intrauterine microfilariae, but not in the adult parasite (Raghavan and Nutman). Another recombinant antigen, WbN-1, was found to share sequences with *B. malayi*, but not other filarial DNA preparations. By immunologic reactions and immunoelectron microscopy it appears that the recombinant WbN-1 represents a gene that codes for filarial myosin (Raghavan, Nutman and collaborators at New England Biolabs). Several recombinant antigens have been identified from expression

libraries of the *Loa loa* parasite. They appear to be specific for this parasite and are being characterized further (Klion and Nutman).

TREATMENT OF FILARIASIS

Continued trials of Ivermectin for treatment of lymphatic filariasis. Through the support of W.H.O., trials of the new drug, Ivermectin, are continuing for treatment of lymphatic filariasis caused by *W. bancrofti*. These trials have been carried out in Madras, India, and in Recife, Brazil, and are part of a 10-center network of investigators around the world. A principal aim of the trials is to determine the optimal regimen using one or two oral doses of ivermectin to clear microfilariae for prolonged periods of time (Ottesen).

Albendazole for *Loa loa*. As a follow-up to earlier studies on immune response to *Loa loa* infection carried out last year in Benin, West Africa, a trial of albendazole treatment was carried out. The reasons to try another drug besides DEC is because some infections with *Loa loa* are refractory, and DEC can cause severe, and even fatal, reactions in microfilaremic patients. Forty heavily microfilaremic patients were enrolled and treated without side effects, but results will not be known for another four months (Nutman, Klion and collaborators in Benin).

Host-Parasite Relations Section

MOLECULAR BIOLOGY AND IMMUNOLOGY OF GIARDIA

Molecular structure and frequency of variant specific proteins (VSPs) in *Giardia*. Analysis of two complete sequences of variant specific proteins (VSPs) from two separate isolates (GS and WB) made it possible to identify two oligonucleotide sequences, one common to all VSPs and the other specific to one VSP (H7). Quantitative comparison of the signal obtained after hybridization of both oligonucleotides to known quantities of a plasmid which contains one copy of each, and to H7 DNA, indicated that there were between 130 and 150 VSP genes present in H7. Further calculations estimated that there are four copies of each gene. Therefore, in this clone there appear to be 30-40 different VSPs. With other methods of estimating the number of VSPs, a range of values from 20 to 184 was obtained (Nash and Mowatt of LPD and Banks and Alling of OSD). The sequences of two VSPs were determined and found to be structurally related but antigenically distinct. VSPs have a common 3' terminal region, so if this portion of the molecule is antigenic, it might provide the basis for a *Giardia* vaccine (Mowatt and Nash).

Immunology and other properties of *Giardia*. Electron microscopic studies showed that VSP was localized to a layer covering the surface of most *Giardia*. However, a small proportion of trophozoites of any given clone fail to react with the approximate monoclonal antibody and presumably lack the VSP (Pimenta and Nash). When strain H7 *Giardia* was used to infect nude mice, it underwent antigenic variation. However, SCID mice, did not exhibit antigenic variation when infected with strain H7, suggesting that intestinal antibodies are responsible for loss of the initial VSP at around day 14 post infection (Nash in collaboration with Gottstein at Univ. of Zurich). The possibility that human immunodeficiency virus (HIV) might be able to grow in protozoa was explored. When HIV was exposed to *Giardia* the parasite RNA was spliced and

could be recovered for a limited period of time. When exposed to *E. histolytica*, some surface proteins of HIV were synthesized (Nash of LPD and Aggarwal of NCI).

CYTOKINES AND
SCHISTOSOME
IMMUNOPATHOLOGY

IL-2 blockade produces different effects in *S. japonicum* vs. *S. mansoni* infections in mice. In contrast to the diminution

in size and fibrosis of schistosome egg granulomas seen in *S.*

mansoni infections after treatment with anti-IL-2 Mabs, no such

effects were seen in *S. japonicum* infections. Interestingly, spleen cells from treated animals showed a dissociation of Th2 type lymphocyte function, with increased IL-4 and decreased IL-5 secretion. These effects were reflected in increased serum IgE levels and decreased blood and tissue eosinophilia in treated animals (Xu, Sher and Cheever). The effects of various cytokines on tissue eosinophilia and on resistance to challenge infections in a different parasite system--*Nippostrongylus brasiliensis* (a murine hookworm) are being examined--(Cheever in collaboration with Finkelman and Urban of USUHS). Reevaluation of old data and new experiments on comparative resolution of liver lesions in *S. mansoni* vs. *S. japonicum* infected mice are in progress. Microscopic hepatic fibrosis resolves rapidly after treatment of either species, but resolution of collagen measured chemically takes place more slowly (Cheever in collaboration with Mosimann of DCRT and Fogarty Scholar Andrade).

Malaria Section

MOLECULAR
GENETICS OF
MALARIA
PARASITES

"Closing the ring" on the chloroquine resistance gene of

P. falciparum. The laborious and long-term quest to identify the gene on *P. falciparum* parasites that controls chloroquine resistance continues, but results indicate that the approach is valid. RFLP markers and chromosome mapping is used to analyze

chromosome inheritance patterns in a genetic cross of chloroquine sensitive with a resistant clone of parasite. Thus far, results show that the chloroquine effect is perfectly linked to a single genetic locus on chromosome 7. Chromosome restriction mapping and crossover analysis place this locus within a 200 kb segment of the chromosome. Several approaches are being used to dissect the resistance locus, such as cloning transcribed sequences for the locus into *E. coli* to examine them for features expected of the resistance gene. Another approach is to make artificial chromosomes in yeast that span the resistance locus. A reagent for labelling membrane-associated transporters may also be useful (Wellems, Peterson, and Walker-Jonah of LPD and Ravetch of Sloan-Kettering and Ravin of NIADDK).

Pyrimethamine and proguanil resistance. Recent work has demonstrated that resistance of malaria parasites against these anti-fol drugs is due to point mutations in the DHFR enzyme of the malaria parasite. The mutations involve changes in amino acids in only three positions of the DHFR enzyme. In some instances a parasite may be resistant to pyrimethamine and sensitive to proguanil and vice versa. In collaboration with Milhous and Andersen at Walter Reed, a structural analog of cycloguanil (the active metabolite of proguanil) has been made which exhibits strikingly different activity against susceptible vs. resistant parasites. The results provide

encouragement for the development of new DHFR inhibitors that could be used for treatment of malaria (Wellems and Peterson of LPD and Milhous and Anderson of Walter Reed).

Genetic switching mechanism for malaria parasite invasion of red cells. The phenomenon described last year is still under study in which a clone of *P. falciparum*, which normally requires sialic acid residues on the red cell membrane for invasion, became able to invade neuraminidase-treated (hence sialic acid deficient) RBCs. It is postulated that some type of switching mechanism under genetic control occurred to permit this. Evidence supporting a genetic-switching mechanism includes the fact that all sub-clones of the original clone which changed are able to invade neuraminidase-treated RBCs, indicating that the capacity for the new invasion mechanism is a property of every member of the population. Current efforts are directed at identifying differentially expressed sequences in the adapted lines by differential colony hybridization and subtraction methods (Dolan and Wellems).

APPROACHES TO MALARIA VACCINES

Transmission-blocking antigens for *P. falciparum*. Antibodies against the 25 kDa surface antigen on ookinetes will block development of the sexual stage of the parasite in the mosquito vector and therefore is a candidate antigen for a vaccine. This antigen, referred to as Pfs 25 has now been cloned and expressed as a recombinant in vaccinia and adenovirus-infected mammalian cells, in bacteria and in yeast. It also has been expressed in stably transfected CHO cells. Experiments with congenic mouse strains with disparate MHC features showed that the recombinant Pfs 25 expressed in vaccinia-infected cells gave an antibody response that was not genetically restricted. Interaction with several commercial groups is underway for production and trials of the recombinant vaccine in humans (Kaslow, Miller, Rawlings, Williamson and Industrial collaborators).

The 40 kDa gamete antigen. This antigen has now been purified to the point where it can be microsequenced. The subcellular localization of this antigen is being studied by EM in gametes (Kaslow and Aikawa of Case-Western).

Glucose-6-phosphate dehydrogenase gene of *Falciparum* malaria. Although it is not a vaccine candidate antigen, this enzyme is interesting in relation to malaria for other reasons. For many years it has been considered that deficiency of this enzyme (G6PD) in people may provide partial protection against malaria. This would imply that the *Falciparum* parasite does not have its own G6PD or that it must depend upon some mechanism of the host to activate the enzyme. By cloning yeast G6PD gene and the use of PCR reactions, it was possible to amplify a fragment of *P. falciparum* DNA and show that the parasite has its own G6PD enzyme. It is planned to produce a recombinant parasite enzyme and test potential inhibitors (Shahabuddin and Kaslow).

Incorporation of malaria antigens into *Salmonella* and BCG. As reported last year, one approach to screen for protective antigens of the malaria parasite is to incorporate constructs of parasite protein into recombinant *Salmonella* which are then inoculated into mice that are challenged with the malaria parasite for evidence of protection. The recombinant *Salmonella* is avirulent and does not persist in the host, but the screening system must utilize a murine malaria parasite. Thus far,

500 constructs have been screened without evidence of protection. A *P. falciparum* surface antigen has been cloned into recombinant *Salmonella*, with plans to test constructs in *Aotus* monkeys. An attempt will also be made to introduce the *P. falciparum* gene into BCG organisms. Such a recombinant experimental vaccine could also be tested in *Aotus* monkeys, and it would be possible to compare two live vector systems, *Salmonella* and BCG (Miller, Kumar, McCutchan, Krettli and collaboration with Ager at University of Miami, Collins at CDC, and Bloom and Flynn at Albert Einstein Medical Center in New York).

Red cell receptors involved in merozoite invasion by the malaria parasite. The genes which constitute the binding proteins for the Duffy blood group antigen have been identified. There is a single gene in *P. vivax* and a family of three genes for *P. knowlesi*. Problems have been encountered in making recombinant proteins produced by these genes, so that specific antibodies to the ligands can be produced by these genes so that specific antibodies to the ligands can be produced (Miller, Adams, Fang and Hudson).

New methodology is being developed for identifying parasite surface proteins involved in recognition of red cell receptors, formation of the parasite-red cell junction, and invasion. A photoactivatable isotope shows some promise of being useful, and merozoites can be labeled with calcium and pH sensitive fluorescent dyes (Miller and Ward).

Attenuated malaria parasites as vaccines. In the same way as mutant *Salmonella* are being studied as avirulent vaccines, attenuated malaria parasites represent another possible approach to a vaccine. Nonreverting mutants of *P. falciparum* have been generated by exposure to gamma irradiation. Auxotrophic mutants which require aromatic and pyrimidine metabolites have also been developed (McConkey and McCutchan).

Parasite Growth and Differentiation Section

MOLECULAR BIOLOGY AND BIOCHEMISTRY OF AMEBAE Further molecular evidence that pathogenic and nonpathogenic *E. histolytica* are distinct species. As reported last year, PCR amplification of small subunit ribosomal RNA (so-called "riboprinting") from pathogenic (P) vs. nonpathogenic (NP) strains of *E. histolytica* showed no differences. 18 "P" and 13 "NP" isolates were studied and a strict correlation between riboprint and previously reported isoenzyme patterns was observed. Attempts to interconvert the two forms failed to produce an alteration in either the isoenzyme or riboprint patterns of the strains. Partial sequence analysis of cloned ribosomal RNA genes showed differences in approximately 2 percent of 780 bases to be scattered throughout the sequence. The possibility that a single "P" or rRNA gene could be amplified during "NP" to "P" conversion was ruled out by failure to detect "P" or rRNA genes in the "NP" genome by use of primers synthesized to specifically amplify "P" rDNA. It was therefore concluded that the previously reported interconversion of "NP" to "P" forms of *E. histolytica* is artifactual and that the forms do indeed represent distinct species.

Some *E. histolytica* isoenzymes may be bacterial in origin. The electrophoretic analysis of isoenzymes originally reported by Sargeant divided *E. histolytica* into 22 different zymodemes;

8 were designated as "P" and 14 as "NP." Experience with the isoenzyme determinations in this lab suggests that many of the isoenzyme bands originally reported are bacterial in origin, and that "P" strains may fall into fewer than the 8 zymodemes first reported (Diamond and Clark).

Chromosome mapping of *E. histolytica*. Large chromosomal-like DNA molecules from *E. histolytica* can be separated by pulse-field electrophoresis. By hybridization with cloned *E. histolytica* genes attempts are being made to identify and map chromosomes of ameba (Mirelman, Clark and Diamond).

Section on Physiology and Biochemistry

STUDIES WITH *T. CRUZI*, *GIARDIA* AND AMEBAE DNA diversity in *T. cruzi*. Differences of up to 40 percent in DNA content were found in naturally occurring variants derived from a single-cell clone of the well-known Y strain of *T. cruzi*. All of the variants have isoenzyme profiles indistinguishable from the parental stock, but do show differences by pulse-field electrophoresis and hybridization with molecular probes (Dvorak). Less striking, but significant, changes in DNA per cell were also observed in clones of *T. cruzi* subjected to environmental effects, such as temperature (Engel, Nozaki and Dvorak). Mutants of *T. cruzi* were produced which were resistant to tubercidin, an adenosine analogue (Nozaki and Dvorak). With some organisms DNA analysis using flow cytometry is not possible. For such situations a relatively simple quantitative image capture and analysis system has been developed, and the software for computer analysis has also been written and verified (Dvorak with collaboration of Mudd of BEIB and Banks and Alling of OSD, NIAID).

Anti-trypanosomal factor from *Pseudomonas*. An improved method was developed for purification of the factor derived from *Pseudomonas fluorescens* which lyses *T. cruzi* (Mercado with collaboration of Fales and Ferrans of NHLBI).

Triose phosphate isomerase of *Giardia*. Fractionation of *G. lamblia* trophozoites showed that the enzyme is localized in the soluble (cytosolic) fraction. Crude preps of the isomerase are highly active and probes and markers are being developed for characterizing genetics of the enzyme (Weinbach and Mowatt).

Immunology of *Strongyloides stercoralis*

Larval protease may be a critical diagnostic target as well as functional element for the parasite. A metallo-protease was demonstrated and characterized in infective larvae (L3s) of this human intestinal nematode. Since inhibitors of the protease, such as EDTA, prevent penetration of L3s through rat skin, activity of the enzyme is undoubtedly necessary to initiate infection. Although multiple antigens are present in L3 materials, Western blot staining for IgE and *in vitro* histamine release assays indicate that the protease is a potent allergen and may be an important component of the immediate hypersensitivity skin test antigen (Brindley, Neva and McKerrow of University of California at S.F.).

Strongyloidiasis as an opportunistic infection. Hyperinfection syndrome and fatal outcome have historically been associated with various forms of immunosuppressive therapy. To our surprise no evidence of complications or hyperinfection with this parasite was found in HIV positive or AIDS patients in Zaire. However, there is increasing evidence that certain patients infected with a different, but related, retrovirus, HTLV-1, can develop serious disease from strongyloidiasis, and cannot be treated successfully. A humoral immune response, especially involving IgE, appears to be more important than a cell-mediated immune response (Neva and C. Brown of NIAID, Perriens of Project SIDA, and collaborators in Jamaica and McFarlin of NINDS and Waldman of NCI).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00094-32 LPD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <i>Entamoeba histolytica</i> : Molecular taxonomy; Genetic Mechanisms of Virulence		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)		
PI:	L.S. Diamond	Section Head LPD, NIAID
Others:	C.G. Clark	IRTA Fellow LPD, NIAID
	A. Bhattacharya	Guest Worker LPD, NIAID
	S. Bhattacharya	Guest Worker LPD, NIAID
	D. Mirelman	Fogarty Scholar FIC
	E.C. Weinbach	Section Head LPD, NIAID
COOPERATING UNITS (If any) Department of Biophysics, Weizmann Institute of Science (David Mirelman)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Parasite Growth and Differentiation		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <p> The question of whether pathogenic 'P' and nonpathogenic 'NP' isolates of <i>E. histolytica</i> constitute one or two species was examined using isoenzyme analysis and 'riboprinting.' A strict correlation between isoenzyme and riboprint patterns was observed for the two forms. Attempts to interconvert the forms failed to produce an alteration in either the isoenzyme or riboprint pattern. Partial sequence analysis of cloned ribosomal RNA genes detected differences at approximately 2% of 780 bases compared. Primers synthesized to specifically amplify 'P' rDNA did not detect 'P' rRNA genes in the 'NP' genome ruling out the possibility a single 'P' rRNA gene is amplified during 'NP' to 'P' conversion. We conclude the reported interconversion of 'NP' to 'P' forms of <i>E. histolytica</i> is artifactual and the forms represent distinct species. The use of riboprinting for identification and phylogenetic analyses of other protozoa was examined using the trypanosomatid genus <i>Crithidia</i>. Data obtained showed the technique to be of phylogenetic value revealing a coevolution of host and parasite groups, and an unexpected close relationship between the genera <i>Crithidia</i> and <i>Leishmania</i>. Sargeant, on the basis of the electrophoretic analyses of four isoenzymes, divided <i>E. histolytica</i> into 22 zymodemes. We examined several of his cloned isolates said to represent 7 zymodemes and found them to fall into three zymodemes. We have evidence the discrepancies between our data and those of Sargeant's are due to his failure to use adequate bacterial controls and as a result misinterpreted many of the bacterial bands as amebal in origin. Separation of large chromosome-like DNA molecules from <i>E. histolytica</i> was achieved by pulse field electrophoresis. At least nine distinct bands were observed with the largest comigrating with Malaria chromosome XIV (3.2 Mb). Identification of the chromosomes and mapping is currently being studied by hybridization with a number of cloned <i>E. histolytica</i> genes. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00097-33 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.I. Mercado	Research Physiologist	LPD, NIAID
Others:	H.M. Fales	Chief, Laboratory of Chemistry	IR, CH, NHLBI
	V.J. Ferrans	Chief, Ultrastructure Section	IR, PA, NHLBI

COOPERATING UNITS (if any)

Waters Life Science Application Laboratory, Fairfax, Va. (M.P. Strickler and J. Stone)
Department of Chemistry, Georgetown University, Washington, D.C. (G. Hammer)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

Studies on the purification of a lytic anti-trypanosomal factor (ATF-II) from the bacterial species, *Pseudomonas fluorescens*, were continued. A purification procedure was developed employing reversed-phase high performance liquid chromatography and was the subject of a patent application which is being processed for review by the U.S. Patent Office. Mass spectroscopic and NMR studies on the structural characterization of the active compound are being continued. The determination of chemical structure is essential before studies on synthesis are initiated and pharmacological and toxicological properties are examined employing *Trypanosoma cruzi* infections.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00098-35 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

P.I.:	E.C. Weinbach	Section Head	LPD, NIAID
Others:	L. Levenbook	Research Chemist	LPB, NIDDK
	L. Diamond	Research Zoologist	LPD, NIAID
	M. Mowatt	Senior Staff Fellow	LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892.

TOTAL MAN-YEARS	4.0	PROFESSIONAL	2.0	OTHER	2.0
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The study of the binding of tricyclic antidepressant drugs, imipramine and chlorimipramine to trophozoites of *Giardia lamblia* was completed this year. Interaction of these drugs with the parasite differs markedly from their interaction with mammalian cells. *G. lamblia* has a large number of binding sites in contrast to mammalian cells, and the binding kinetics shows very weak binding. There is no evidence for specific binding receptors in the parasite as is present in mammalian cells. This may be a reflection of the primitive position of *G. lamblia* on the evolutionary tree of eukaryotic cells.

Collaborative studies, initiated last year with Dr. Mowatt, examined the biochemical and genetic characteristics of a key glycolytic enzyme triosephosphate isomerase. Initial studies have now localized the enzyme in the soluble (cytosolic) fraction of *G. lamblia* trophozoites. It appears to be a true soluble enzyme not associated with any glycosomal-like structures as in other parasitic protozoa. Genetic probes and markers for this enzyme are being developed by Dr. Mowatt.

Collaborative studies with Dr. Diamond of the respiratory metabolism of *Entamoeba* confirmed our previous observation that only the reptilian species exhibited a marked stimulatory effect of Mn^{2+} on NAD(P)H oxidase. This observation may correlate with the distinct differences in molecular taxonomy found among these species of *Entamoeba*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00099-21 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biophysical Parasitology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	J. A. Dvorak	Res. Microbiologist	LPD, NIAID
Others:	J. P. McDaniel	Biologist	LPD, NIAID
	T. Nozaki	Fogarty Fellow	LPD, NIAID
	S. Banks	Senior Staff Fellow	OSD, NIAID
	D. Alling	OSD, NIAID	
	D. Stephany	BRB, DIR, NIAID	
	J. Hooley	BRB, DIR, NIAID	
	C. P. Mudd	Senior Engineer	ACE, BEIB

COOPERATING UNITS (If any) Biomedical Engineering and Instrumentation Branch, DRS; Office of the Scientific Director, NIAID; Laboratory of Applied Studies, DCRT; Biological Resources Branch, Division of Intramural Research, NIAID

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

This project is concerned with (a) analyses of the genetic diversity of *Trypanosoma cruzi* and its implications to the epidemiology, course, and diagnosis of Chagas' disease; (b) the development of high resolution flow cytometry instrumentation for biomedical research; and (c) the utilization of flow cytometry and low light level video microscopy for the analyses of infectious agents. We have demonstrated that DNA variants of *T. cruzi* occur at low frequency from a single-cell isolate clone stock. These variants are being characterized. Environmental parameters such as temperature can destabilize the DNA of *T. cruzi* resulting in the appearance of variant stocks. Drug and radiation-induced *T. cruzi* mutants have been produced which are resistant to tubercidin, an adenosine analogue. These mutants are being characterized in an attempt to determine the genetic basis for drug resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00102-17 LPD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Disease Caused by Infection with Intracellular Parasites		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	F. A. Neva Chief	LPD, NIAID
Others:	D. Sacks Research Microbiologist	LPD, NIAID
	C. Karp NRSA Fellow	LPD, NIAID
	A. Cooper Visiting Fellow	LPD, NIAID
COOPERATING UNITS (If any) Ministry of Health, Tegucigalpa, Honduras (C. Ponce); Inst. de Salubridad y Enfermadades Trop., Mexico City (O. Velasco); Dept. Biol, Youngstown U, Ohio (R. Kreutzer); Yale U Sch of Pub. Hlth, Dept. of Epidemiology, New Haven, CT (D. McMahon-Pratt)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Cell Biology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <p>The emphasis of this project is still mainly upon the different clinical forms of leishmanial infection in humans, their immune response to leishmanial antigens, and characteristics of the causative parasites.</p> <p>Six parasitologically proven new cases of cutaneous leishmaniasis were diagnosed and treated during the last year. Several additional patients diagnosed elsewhere were evaluated, and two patients with previous leishmaniasis were leukophoresed to obtain peripheral blood mononuclear cells for research purposes.</p> <p>A profile of immunologic studies was carried out on a small group of patients in Honduras with the atypical form of cutaneous leishmaniasis caused by <i>L.D. chagasi</i>. A patient with diffuse cutaneous leishmaniasis (DCL) was treated with Pentostam plus Interferon-gamma.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00108-20 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies on the Immunobiology of Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI:	L.H. Miller	Head, Malaria Section	LPD, NIAID
	S. Kumar	Visiting Associate	LPD, NIAID
Others:	T. McCutchan	Senior Scientist	LPD, NIAID
	Glenn McConkey	NRC Fellow	LPD, NIAID
	Antoniana Krettli	Guest Worker	LPD, NIAID
	Peter Perlmann	Fogarty Scholar	LPD, NIAID
	Hedvig Perlmann	Guest Worker	LPD, NIAID

COOPERATING UNITS (if any)

W. Weiss, Naval Med. Res. Inst.; J.A. Berzofsky, Sr. Scientist, Metabolism Branch, NCI; R. Houghten, M. So, and F. Heffron, Univ. of Oregon, Portland; M.F. Good, QIMR, Brisbane, Australia; Arba Acar, U. of Miami; William E. Collins, CDC, Atlanta, Barry Bloom and Joann Flynn, Albert Einstein Medical Center, Bronx, NY.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.2

PROFESSIONAL

1.2

OTHER

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type Do not exceed the space provided)

Immunity to asexual erythrocytic parasites in certain rodent malarias is dependent on cell mediated mechanisms that are dependent on CD4+ T cells and the spleen and are independent of antibody. Such immunity can be induced by Salmonella and malarial antigens. The present studies are designed to identify those proteins and T cell epitopes that will lead to protection. In addition, we are developing studies to test constructs in Salmonella and BCG that will lead to protection against *Plasmodium falciparum* in *Aotus* monkeys.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00161-14 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.E. Nash	Medical Officer	LPD, NIAID
Others:	F.A. Neva	Chief	LPD, NIAID
	A.W. Cheever	Assistant Chief	LPD, NIAID
	M.M. Mowatt	Staff Fellow	LPD, NIAID
	B. Gottstein	Institute of Parasitology, Univ. of Zurich,	
		Switzerland	

See below

COOPERATING UNITS (if any)

A. Aggarwal, NCI

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

1.5

PROFESSIONAL

0.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

G. Shearer	LPD, NIAID
S. Banks, D. Alling	Office of Scientific Director NIAID
P. Pimenta	LPD, NIAID
G. Harriman, W.S. Strober	LCI, NIAID

The number of variant specific proteins (VSPs) in one *Giardia* clone was estimated to be between 130 and 150. Four copies of each gene appear to present and therefore between 30-40 different VSPs exist in this clone.

Electron microscopy studies showed some *Giardia* lack a surface coat where VSPs are localized.

Giardia infected athymic mice undergo antigenic variation but SCID mice do not suggesting non-cellular processes are responsible for loss of the initial VSP.

HIV can enter and survive in *Giardia*; however, it is not clear if viable HIV are produced.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00162-15 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	D.M. Dwyer	Supervisory Microbiologist	LPD,NIAID
Others:	G.O. Gbenle	Intl. Res. Fellow, FIC	LPD,NIAID
	D.J. Mallinson	Visiting Fellow, FIC	LPD,NIAID
	V.H. Mann	IRTA Fellow	LPD,NIAID
	C. McCarthy-Burke	Biologist	LPD,NIAID
	D. Zilberstein	Visiting Scientist, FIC	LPD,NIAID

COOPERATING UNITS (if any)

Dept. Zool., Univ. Glasgow (P.A. Bates); Depts. Biochem. and Microbiol., Univ. Victoria (R.W. Olafson, T.W. Pearson & D.L. Tolson); Dept. Biochem., Univ. Kentucky (S. Turco); CBER, DBB, FDA (H. Nakhasi).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology & Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4.0

PROFESSIONAL

3.4

OTHER

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The cell biology of *Leishmania* is investigated as a model of both intra- and extracellular parasitism. Emphasis is placed on characterizing the biochemical and physiological functions of its surface membrane (SM) and secreted components toward defining their roles in parasite survival and development.

Leishmania promastigotes possess a number of SM transport systems for the active accumulation of certain essential amino acids and nucleosides. One of these, a putative SM adenosine transporter was identified *in situ* in *L. donovani* promastigotes using several radiolabeled, affinity-binding, cross-linking reagents. This externally oriented SM protein has an apparent molecular weight of 30 kDa is currently being characterized. Both molecular and immunochemical approaches are being used to identify genes coding for the 2 leishmanial L-proline transport systems. *Leishmania* and *Crithidia* were both shown to use S-adenosyl-methionine as a protein methylating agent. In both of these trypanosomatids, methylation appears to be a mechanism for modifying/regulating the activity of certain SM proteins *in situ*. The SM 3'-nucleotidase of *L. donovani* promastigotes was purified to homogeneity. The substrate specificity, metal cation requirements and inhibitors of this 38 kDa glycoprotein were characterized. Three separate glycosylation events were identified during the biosynthesis of the leishmanial secretory acid phosphatase (SACP). The last of these includes the Golgi-based addition of: $[\text{PO}_4\text{-6Gal}\beta\text{1,4Man}]_n$, the repetitive, constitutive epitope of the SM lipophosphoglycan (LPG). Terminal stages in LPG biosynthesis were also shown to occur in the Golgi compartment. Genetic analyses of subtractive libraries is being used to identify genes involved in LPG expression. cDNA clones are being sequenced to identify the full length copy of the SACP gene. *In vitro* cultivated amastigotes are being used as a model of parasite gene regulated differentiation and development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00197-12 LPD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunoregulation and immune recognition in filariasis and non-filarial diseases.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T.B. Nutman	Senior Investigator LPD, NIAID
Others:	E.A. Ottesen	Senior Investigator LPD, NIAID
	C.L. King	Senior Staff Fellow LPD, NIAID
	S. Mahanty	NRSA Fellow
	A. Klion	Medical Staff Fellow LPD, NIAID
COOPERATING UNITS (if any) Department of Allergy and Clinical Immunology, Univ. of Montreal, Montreal, Canada (G. Delespesse); Dept. of Onchocerciasis, SNEM, Guatemala City, Guatemala (G. Zea-Flores), Univ. National de Benin (Dr. Massouboudgi), Anna Univ., Madras India (Dr. K. Jayaraman)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Clinical Parasitology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	2.5	PROFESSIONAL: 1.7 OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) The purpose of this project is to delineate the mechanisms involved in regulating the humoral and cellular responses in patients with filariasis and other disease states. Immunoregulatory studies have examined the phenomenon of antigen-specific anergy in microfilaremic patients by showing this anergy to be a result of a diminished frequency of proliferating cells to parasite antigen. <i>In vitro</i> models of parasite-antigen driven antibody production along with recombinant lymphokines and neutralizing antibodies have been used to understand the signals necessary to regulate antibody production in response to parasite antigen at a molecular level.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00208-11 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	Thomas F. McCutchan	Microbiologist
Others:	G. McConkey	NRC Fellow
	S. Gigliotti	Technician
	W. White	Guest Worker
	C. Mathiopoulos	Fogarty Fellow
	G. Lanzaro	Guest Worker

COOPERATING UNITS (If any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

3.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided..)

Our laboratory is involved in two central projects. We are producing attenuated lines of human and rodent malarias which should prove useful in the study of both the biochemistry of parasites and the development of immunity to parasites. We have also been characterizing the ribosomal RNA of different malarial parasites. Information from these studies has important ramifications for the development of malaria diagnostics, the understanding of evolutionary relationships among the different malarial species and the understanding of control of the parasite's developmental cycle.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00240-10 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: D. C. Kaslow

Senior Staff Fellow

LPD, NIAID

L. H. Miller

Head, Malaria Section

LPD, NIAID

Others: D. Rawlings, Guest Worker, LPD, NIAID; K. Williamson, Guest Worker, NIAID; M. Shahabuddin, Guest Worker, LPD, NIAID; D. Keister, Biologist, LPD, NIAID, Chiron Corp, Emeryville, CA (P. Barr and I. Bathurst); Wyeth Labs, Radnor, PA (P. Hung); Catholic U, Nijmegen, Netherlands (J. Schoenmakers and T. Ponnudurai); Pasteur Inst, Paris, (G. Langsley); Hazelton Labs, Vienna, VA (S. Harshman); Scripps Clin. Res. Fdn., La Jolla, CA; J. Coligan, Branch Chief, BRB, NIAID; S. Isaacs, Medical Staff, B. Moss, Lab Chief, LVD, NIAID

COOPERATING UNITS (if any)

See attached

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIH, NIAID, Bethesda, MD 20892

TOTAL MAN-YEARS

6.5

PROFESSIONAL

3.5

OTHER

3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Our goals are, first, continue development of a 25 kDa sexual stage surface antigen, Pfs25, as a potential vaccine candidate; second, clone the genes for the other known target antigens of transmission blocking immunity; third, identify new target antigens on sexual stage parasites; and finally, define the molecular mechanisms involved in fertilization of malarial parasites. In addition, we are studying the parasite glucose-6-phosphate dehydrogenase (G6PD) enzyme and gene and its role in the protection afforded by G6PD deficiency in humans.

Previously we had cloned the gene encoding Pfs25, a prime candidate antigen for a transmission blocking vaccine. Pfs25 has now been expressed in bacteria, yeast, vaccinia and adenovirus infected mammalian cells, transiently transfected COS cells, and stably transfected CHO cells. Data from mice and Aotus monkeys immunized with vaccinia-produced Pfs25 are very encouraging: sera from mice and monkeys inoculated with live, recombinant vaccinia block transmission of malaria. We have also found that mice and monkeys immunized with purified yeast-produced Pfs25 also acquire transmission blocking immunity. Several adjuvant systems have been examined and alum or DPT has been found to be adequate. Cloning the other target antigens has been a problem, although we now believe that we have cloned, sequenced, and expressed in *E. coli* Pfs40, a potential target antigen based on immunogenetic data. A microgamete (male) specific monoclonal antibody has been developed and may provide the entre we need to understand the molecular mechanisms involved in fertilization in the malaria parasite.

Finally, we have demonstrated that the malaria parasite expresses G6PD constitutively, and independently of the G6PD status of the host. We have cloned the *P. falciparum* G6PD gene.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00241-10 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	L.H. Miller	Section Head	LPD, NIAID
Others:	J. Adams	Staff Fellow	LPD, NIAID
	D. Hudson	Microbiologist	LPD, NIAID
	T. Wellems	Sr. Staff Fellow	LPD, NIAID
	D. Kaslow	Staff Fellow	LPD, NIAID
	X. Fang	Visiting Fellow	LPD, NIAID
	G. Ward	Visiting Associate	LPD, NIAID

COOPERATING UNITS (if any)

WRAIR, Washington, DC (F. Klotz and J.D. Haynes); Hazelton Laboratories, Vienna, VA (J. Rener); Case Western Reserve Univ., Cleveland, OH (M. Aikawa)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS

4.3

PROFESSIONAL

4.3

OTHER

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage upon which immunity may work to block invasion. Thus, merozoite surface components are of interest because of their role in erythrocyte recognition and as antigens for induction of protective immunity. We are identifying *P. knowlesi*, *P. vivax* and *P. falciparum* receptors for attachment to monkey and human erythrocytes. To understand the molecular basis for this variation we are studying one antigen on the merozoite surface that undergoes antigenic variation. The components in the junction and the signaling after merozoites make contact with erythrocytes is also under study.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00246-09 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Studies of the Genome and Surface of *Schistosoma mansoni*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Section Head	LPD/NIAID
Others:	E.J. Pearce	Visiting Associate	LPD/NIAID
	C. Champion	Special Volunteer	LPD/NIAID
	I. Oswald	Special Volunteer	LPD/NIAID
	J. Actor	IRTA Fellow	LPD/NIAID
	P. Brindley	Visiting Associate	LPD/NIAID

COOPERATING UNITS (if any)

Molecular Vaccines, Inc., Gaithersburg, MD (V. de la Cruz)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS

2.5

PROFESSIONAL

2.0

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The aim of this project is to characterize schistosome molecules relevant to immunity and pathogenesis, to analyze the molecular basis of physiologically important parasite processes which could serve as targets for intervention, and to develop and test recombinant vaccines. Progress was achieved in the following areas:

- A. Testing of BCG-paramyosin recombinants. The immune response stimulated by a vaccine recombinant produced by cloning paramyosin into BCG was characterized.
- B. Identification of recombinant clones expressing epitopes reactive with vaccine T cells. Clones from an expression library were screened against T cells from vaccinated mice in order to identify potential new immunogens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00248-10 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.W. Gwadz	Senior Investigator	LPD, NIAID
	L.H. Miller	Section Chief	LPD, NIAID
	T.F. McCutchan	Senior Investigator	LPD, NIAID

Other: (see attached)

COOPERATING UNITS (if any)

Inst. of Parasitology, U. of Rome (Dr. M. Coluzzi); Inst. of Pathology, Case Western Reserve School of Med. (Dr. M. Aikawa); National Med. School, Bamako, Mali (Dr. Y. Toure); U. of MD Biotechnology Inst. (Dr. R. Colewell).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

11.5

PROFESSIONAL:

9.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The biology of anopheline mosquitoes is being studied in relation to the capacity of these vectors to transmit malaria. Molecular, biochemical, and immunological studies are seeking to describe mechanisms of malaria parasite development in the mosquito host with emphasis on the behavior of ookinetes, oocysts, and sporozoites. Probes are being developed to facilitate characterization of cytogenetically distinct populations of *Anopheles gambiae* in Mali, West Africa.

Studies on the composition of the mosquito larval diet seek to identify microorganisms which might prove useful as carriers of biologically active larvicidal agents.

Systems for cloning and transposing genes into mosquito germ lines are being developed to assist in generating vector species unable to transmit malaria parasites in nature. Parasiticidal mechanisms from within mosquitoes and from other organisms are being evaluated for introduction into vector species.

The ability to identify, clone, and transpose genes which regulate refractory mechanisms should permit development of mosquito populations incapable of transmitting malaria for eventual use in malaria control schemes.

Other:	R.K. Sakai	Expert (resident in Mali)	LPD, NIAID
	D.C. Kaslow	Med. Staff Fellow	LPD, NIAID
	G.C. Lanzaro	Guest Researcher	LPD, NIAID
	C. Mathiopoulos	Vis Fellow	LPD, NIAID
	M. Huber	Visiting Fellow	LPD, NIAID
	A. Warburg	Visiting Associate	LPD, NIAID
	M.G. Touray	Visiting Fellow	LPD, NIAID
	D.L. Bryant	Guest Researcher	LPD, NIAID
	H.G. Coon	Senior Investigator	LG, NCI
	K. Mizuuchi	Senior Investigator	LMB, NIDDK
	M.S. Lee	Guest Researcher	LMB, NIDDK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00251-10 LPD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Studies on Schistosomiasis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	A. Sher	Section Head LPD, NIAID
Others:	E. J. Pearce	Visiting Associate LPD, NIAID
	S. L. James	Program Officer MIDP, IRP, NIAID
	A. W. Cheever	Assistant Chief LPD, NIAID
	I. Oswald	Special Volunteer LPD, NIAID
	M. Williams	Special Volunteer LPD, NIAID
	J. Berzofsky	Section Head MEI, NCI
COOPERATING UNITS (if any) Uniformed Services University of the Health Sciences (F. Finkelman)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Immunology and Cell Biology Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The aim of this project is to study mechanisms of immunity, immunoregulation, immune evasion and immunopathology in schistosomiasis with the ultimate goals of developing an experimental vaccine suitable for human trials as well as understanding the pathogenesis of disease.</p> <p>In work completed this year, mice infected with <i>S. mansoni</i> were demonstrated to have a selective defect in their capacity to mount Th1 cytokine responses to a foreign antigen. Moreover, IL-10 a cytokine previously shown to be produced by these animals, was demonstrated to inhibit the capacity of activated macrophages to kill schistosome larvae.</p> <p>Studies on immunopathogenesis revealed that IL-2 depletion reduces both granuloma formation and fibrosis in <i>S. mansoni</i> infected mice while downregulating IL-5 but up-regulating IL-4 synthesis. The pattern of cytokine production in <i>S. japonicum</i> infected mice was found to closely mirror that previously observed in <i>S. mansoni</i> infected animals.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00253-10 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Immunological Responses to Filarial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	T.B. Nutman	Senior Investigator	LPD, NIAID
	C. King	Medical Staff Fellow	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID
	N. Raghavan	Fogarty Fellow	LPD, NIAID
See below	E. Lobos	Fogarty Fellow	LPD, NIAID

COOPERATING UNITS (If any)

Indian Cncl of Med Res, Madras, India (S Tripathy, V Kumaraswami); Anna Univ, Madras, India (K Jayaraman); Dept of Hlth, Guatemala (G Zea-Flores); CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Coutinho); U. of Minnesota (E Holland); U.

LAB/BRANCH of Alabama (D.O. Freedman)

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.9

PROFESSIONAL:

1.9

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

S. Mahanty, NRSA Fellow, LPD, NIAID; J. McCarthy, Visiting Associate, LPD, NIAID; F.A. Neva, Chief, LPD, NIAID; R.B. Nussenblatt, Clinical Director, CB, NEI

The purpose of this project is to define the humoral and cellular immune responses that relate to immunodiagnosis, immunopathology, and protective immunity of patients with lymphatic filariasis, onchocerciasis, and loiasis.

Serodiagnosis of new or pre-patent onchocerciasis infections has been made feasible through the use of a purified recombinant onchocercal antigen (OV16). Serologic assays for filarial infection based on IgG4 antibody detection have been shown to have enhanced specificity, but such antibodies require 6-9 months to develop despite the strong stimulation of chronic helminth infection.

Histopathologic and serologic evidence implicates the eosinophil and its granule proteins as a primary determinant of post-treatment reactions in onchocerciasis and in the pulmonary pathology of the Tropical Eosinophilia syndrome in bancroftian filariasis; IL-5 appears to be the primary mediator of the eosinophil responses.

Populations with bancroftian filariasis or onchocerciasis have been examined to define immunologic parameters that distinguish "naturally immune" from infected individuals. A 43kD protein from infective larvae that may be a protective immunogen for bancroftian filariasis has been cloned and sequenced; its practical usefulness is under further study. Similar studies in onchocerciasis have also identified several differentially recognized molecules that are being purified and evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00255-10 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Immunologic Responses to Non-Filarial Parasitic Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	T.B. Nutman	Senior Investigator	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID

COOPERATING UNITS (If any)

CPqAM/FIOCRUZ, Recife, Brazil (G. Dreyer, A. Coutinho)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.3

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Serial serum specimens from untreated patients with acute schistosomiasis have provided direct evidence that chronic antigenic stimulation is required for IgG4 responses to develop in humans. Despite intense clinical and immunological stimulation, patients were unable to mount specific IgG4 responses for at least 4-9 months after becoming infected; the rapidity of the onset of such responses was directly proportional to the intensity of infection. The kinetics of IgE and IgG4 responses appear similar and distinct from those of the other IgG subclasses.

The tropical pulmonary eosinophilia syndrome (TPE) is generally caused by filarial parasites, but recent evidence indicates that strongyloides infection may also induce a syndrome clinically indistinguishable from TPE. Detailed immunologic analysis is underway to define serodiagnostic means to differentiate between these two clinical syndromes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00256-10 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Biology of Leishmania Promastigotes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Sacks Senior Investigator LPD/NIAID

Other: T. Brodin Guest Researcher LPD/NIAID
P. Pimenta Guest Researcher LPD/NIAID
C. Karp Guest Researcher LPD/NIAID

COOPERATING UNITS (if any)

Dr. Sam Turco, Dept. of Biochemistry, University of Kentucky
Dr. Malcolm McConville, Dept. of Biochemistry, University of Dundee, Scotland

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology Section

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:

3.2

PROFESSIONAL:

3.2

OTHER:

.

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

During growth within the sand fly and within axenic culture, *Leishmania* promastigotes undergo differentiation from a dividing noninfective stage to a resting infective or metacyclic stage which is uniquely adapted for life in the vertebrate. For some species of *Leishmania* (*L. donovani* and *L. major*) this development is accompanied by a substantial modification of the surface lipophosphoglycan (LPG) which is the major surface glycoconjugate of these cells. The LPG is densely organized into a glycocalix which effectively masks other surface antigens and which is not expressed on intracellular amastigotes. The complete structures of log phase and metacyclic LPGs of both species have now been determined. The developmental modifications include an elongation of the molecule due to a 2-3 fold increase in the number of phosphorylated oligosaccharated units expressed, and a change in the composition of some of these units. In each case the structural polymorphism is expressed as a loss of terminal galactose residues on the LPG. The effect of this developmental change on the binding of promastigotes to midgut epithelial cells was investigated. An average of 8400 log phase promastigotes bound per midgut, while metacyclic binding was negligible. The binding of log promastigotes was completely inhibited by log phase LPG, but not by metacyclic LPG. The specific oligosaccharide units which mediate the binding is now being studied. In related studies, it was found that the presence of anti-LPG antibodies within an infective bloodmeal inhibited the development of transmissible infections within the sand fly midgut. Thus, immunization of reservoir hosts with LPG may form the basis of a transmission blocking vaccine for leishmaniasis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00257-10 LPD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunology of Strongyloidiasis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	F.A. Neva	Chief LPD, NIAID
Others:	F.J. Brindley	Guest Researcher LPD, NIAID
	C. Brown	Medical Staff Fellow LIR, NIAID
	Eric Denkers	IRTA Fellow LPD, NIAID
	T. Nutman	Senior Investigator LPD, NIAID
	E. Ottesen and T. Nash	Senior Investigators LPD, NIAID
	D.E. McFarlin	Chief NI Branch, NINDS
COOPERATING UNITS (If any) Univ. of California at S.F., Dept. Pathology (J. McKerrow); SEMA, Inc., Rockville (T. Moskal); Univ. of the West Indies, Jamaica (Prof. O. Morgan, Ralph Robinson and S. Terry); Project SIDA, Kinshasa, Zaire (J. Perriens); Univ. of Mass., Dept. of		
LAB/BRANCH Family Medicine (P. Gann) Laboratory of Parasitic Diseases		
SECTION Cell Biology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 0.8	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="display: flex; justify-content: space-between; margin-top: 10px;"> T.W. Waldman Chief Met. Branch, NCI </div> <p style="margin-top: 20px;">This project involves both clinical studies of humans infected with the intestinal nematode, <i>Strongyloides stercoralis</i>, and laboratory analysis of parasite antigens and immunologic responses to them. The parasite is of particular interest because of its unusual biologic properties (no parasitic male worm, free-living stage, etc.), and the fact that it can produce serious, even fatal, disease in certain immunosuppressive states.</p> <p style="margin-top: 20px;">We continue to have problems in purification of the larval protease which would probably be the optimum diagnostic skin test antigen (immediate reaction). Western blotting with IgE and histamine-releasing activity are the two types of assays for allergenic activity, but there are probably multiple antigens present capable of these properties.</p> <p style="margin-top: 20px;">The association of strongyloides infection in patients infected with HTLV-1 retrovirus continues to be investigated. Many of these patients have very low total serum IgE levels so their cytokine responses are being examined. The parasite-specific IgE assay (Elisa) appears to be sensitive and specific. Another group of patients with strongyloides infection has become available for study from our collaboration with University of Massachusetts in assessing prevalence of this infection in Cambodian refugees in Lowell, Massachusetts. This study also involves comparative efficacy of treatment with Ivermectin vs. thiabendazole.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00347-09 LPD

PERIOD COVERED

October 1, 1990 - September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies on Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: A.W. Cheever, Head, Host-Parasite Relations, LPD, NIAID

Others: J. Macedonia Bio. Lab. Tech., LPD, NIAID
T. Cox Bio. Lab. Tech., LPD, NIAID
Y. Xu Visiting Fellow, LPD, NIAID
A. Sher Head, Immunology Sect., LPD, NIAID

COOPERATING UNITS (if any)

Department of Medicine, USUHS (Fred Finkelman) and USDA (Joseph Urban).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, Md 20892

TOTAL MAN-YEARS

2.0

PROFESSIONAL

1.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Hepatic fibrosis and the granulomatous response to eggs of schistosome species pathogenic for man are studied in mice in relation to parasitologic parameters of infection. Cytokines play an important role in the genesis and regulation of the size of circumoval granulomas and in the fibrosis associated with them. Treatment of mice with antibodies to IFN- γ has no evident effect on granulomas around *S. mansoni* eggs but decreases the size of the reaction to *S. japonicum* eggs but without affecting hepatic fibrosis. Blockade of IL-2 using monoclonal antibodies against IL-2 and IL-2 receptor decrease both the size and fibrosis of granulomas around *S. mansoni* eggs but have no affect in *S. japonicum* infected mice. IL-2 blockade inhibits IL-5 secretion in vitro and decreases blood and tissue eosinophilia in vivo while IL-4 secretion is increased and serum IgE increases. These latter effects are similar in *S. mansoni* and *S. japonicum* infected mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00350-09 LPD															
PERIOD COVERED October 1, 1990 to September 30, 1991																	
TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.) DNA Analysis of Parasites																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">T.E. Nash Medical Officer</td> <td style="width: 40%;">LPD, NIAID</td> </tr> <tr> <td>Others:</td> <td>R. Adam Assistant Professor, Medicine</td> <td>University of Arizona</td> </tr> <tr> <td></td> <td>M. Mowatt Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td></td> <td>B.Y. Nguyen Medical Staff Fellow</td> <td>LPD, NIAID</td> </tr> <tr> <td colspan="3">see below</td> </tr> </table>			PI:	T.E. Nash Medical Officer	LPD, NIAID	Others:	R. Adam Assistant Professor, Medicine	University of Arizona		M. Mowatt Staff Fellow	LPD, NIAID		B.Y. Nguyen Medical Staff Fellow	LPD, NIAID	see below		
PI:	T.E. Nash Medical Officer	LPD, NIAID															
Others:	R. Adam Assistant Professor, Medicine	University of Arizona															
	M. Mowatt Staff Fellow	LPD, NIAID															
	B.Y. Nguyen Medical Staff Fellow	LPD, NIAID															
see below																	
COOPERATING UNITS (if any) None																	
LAB/BRANCH Laboratory of Parasitic Diseases																	
SECTION Host-Parasite Relations Section																	
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20892																	
TOTAL MAN-YEARS 3.5	PROFESSIONAL 2.5	OTHER 1.0															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided) <table style="width: 100%; border: none; margin-top: 10px;"> <tr> <td style="width: 60%; vertical-align: top;"> J. Moss A. Geber J. Weiss </td> <td style="width: 40%; vertical-align: top;"> NHLBI LCI, NIAID Roche Diagnostics Research </td> </tr> </table> <p style="margin-top: 20px;">The sequences of two variant specific proteins(VSPs) have been determined. They reveal that VSPs, even from unrelated isolates, are compositionally and structurally related. Although identity among VSPs are about 50% they are antigenically distinct. Inexpressible epitopes are not represented in the genome. VSPs have a common 3' terminal region. If host's respond or can be made to respond to this area, vaccination may be possible.</p> <p>The genomic organization of VSP1267 revealed two identical genes arrange tail to tail with an intervening 3kb region.</p> <p>The VSP repetitive epitope recognized by Mab 6E7 in VSPWB170 is present in other isolates, but the sequences of some are different.</p> <p>The analogue of the G-protein ADP-ribosylation factor has been identified and sequenced in WB and GS <i>Giardia</i> isolates. Although there is near identity at the amino acid level, the sequences are only 87% identical indicating significant drift.</p>			J. Moss A. Geber J. Weiss	NHLBI LCI, NIAID Roche Diagnostics Research													
J. Moss A. Geber J. Weiss	NHLBI LCI, NIAID Roche Diagnostics Research																

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00439-07 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies in Human Filariasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.A. Ottesen	Section Head	LPD, NIAID
Others:	T.B. Nutman	Senior Investigator	LPD, NIAID
	C. King	Medical Staff Fellow	LPD, NIAID
	A. Klion	Medical Staff Fellow	LPD, NIAID
	S. Mahanty	NRSA Fellow	LPD, NIAID
	F.A. Neva	Chief	LPD, NIAID

COOPERATING UNITS (if any) Indian Council of Medical Research, Madras, India (S.P. Tripathy, V. Kumaraswami, R. Prabhakar); MGR Medical College, Madras, India (V. Vijayasekaran); Peace Corps Medical Office (C. Waterman); Centro de Pesquisas Aggeu Magalhaes, Recife, Brazil (A.

LAB/BRANCH Coutinho, G. Dreyer); Dept of Health, Coronou, Benin (A. Massougbedji).
Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

Ivermectin has been shown in trials in both South India and elsewhere to be very effective in clearing microfilaremia in patients with bancroftian filariasis. Current studies in South India and Brazil (coordinated with trials elsewhere in the world) are focussed on developing a regimen to optimize the duration of microfilarial clearance.

Recent investigation has indicated that the Tropical Pulmonary Eosinophilia syndrome can be caused by strongyloides as well as by filariae. Work to differentiate these two pulmonary eosinophilic conditions and develop appropriate therapy is underway.

Loiasis in expatriate visitors to endemic areas has been characterized as having marked clinical and immunologic hyperresponsiveness to the filarial parasite. The hypothesized state of hyporesponsiveness, both clinical and immunological, in infected individuals native to the endemic regions has now been demonstrated by studies in Benin, West Africa. A therapeutic trial of albendazole for loiasis is also underway there.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00483-06 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Molecular Genetics of Drug Resistance and Red Cell Invasion in Malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title laboratory, and institute affiliation)

PI:	Thomas E. Wellems	Sr. Staff Fellow	LPD, NIAID
Others:	David Peterson	Research Fellow	LPD, NIAID
	Stephen Dolan	Med. Staff Fellow	LPD, NIAID
	Annie Walker-Jonah	Fogarty Fellow	LPD, NIAID
	Kathleen Creedon	Guest Worker	LPD, NIAID

See below

COOPERATING UNITS (if any)

Sloan Kettering Institute, New York (Dr. J. Ravetch); Laboratory of Cell Biology, NIADDK (Yosef Raviv); Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. (W.K. Milhous, S.L. Andersen); Catholic University, Washington, D.C. (P. Rathod)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

6

PROFESSIONAL

4

OTHER

2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Frederic Gyang	Fulbright Scholar	Univ. of Ghana
Jacqueline Herrfeldt	Coop Student	George Washington, Washington DC
Donald Krogstad	Visiting Scientist	Washington Univ., St. Louis

Our investigations are directed at the mechanisms of drug resistance and red blood cell invasion by the malaria parasite *Plasmodium falciparum*. Chloroquine-resistant strains of *P. falciparum* counter the drug by expelling it rapidly via an unknown mechanism. Genetic linkage analysis has shown that chloroquine resistance is controlled by a single gene or closely linked group of genes within a ~200 kilobase segment on the parasite's seventh chromosome. Characterization of genes within this segment should lead to an understanding of the rapid efflux mechanism. Point mutations in the dihydrofolate reductase (DHFR) gene confer different degrees of resistance to two DHFR inhibitors, pyrimethamine and cycloguanil, depending upon the positions of the mutations and the residues involved. The Asn-108 mutation plays a central role in pyrimethamine resistance. A survey has shown ~90% incidence of this mutation in a wide section of the Brazilian Amazon, consistent with pyrimethamine failure rates. A structural analog of cycloguanil has high activity against pyrimethamine- and cycloguanil-resistant forms of *P. falciparum*, suggesting that it may be possible to develop alternative DHFR inhibitors as drugs. A switch to an alternative pathway of red cell invasion has been detected in a *P. falciparum* clone made to propagate in neuraminidase-treated, sialic acid-deficient red blood cells. The switch may involve a mechanism in which certain genes are activated or rearranged. Differential and subtractive screening methods are being used to search for these genes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00487-05 LPD

PERIOD COVERED

October 1, 1990 - September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Studies on the Quantitative Parasitology of Schistosome Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)

PI: A.W. Cheever, Chief, Host-Parasite Relations Section

Others: J. Macedonia Bio. Lab. Tech., LPD, NIAID
T. Cox Bio. Lab. Tech., LPD, NIAID
J. Mosimann LSM, DCRT

COOPERATING UNITS (if any)

Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil (Zilton Andrade, Fogarty Scholar).
Biomedical Research Institute, Rockville, MD (Fred Lewis).

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS

1.0

PROFESSIONAL

0.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

Present efforts are concentrated on evaluating the role of host reactions on parasitologic parameters (see Z01 AI 00347) and on the development of models for the kinetics of egg laying, egg destruction and egg excretion in *S. mansoni* and *S. japonicum* infections. Accurate assessment of the rate of egg destruction would be important for constructing these models but we have failed to obtain estimates that would appear to be useful for such models. We will proceed with the modeling but without great expectation that we will be able to put useful limits on the rate of egg laying by the parasites. Resolution of hepatic fibrosis after chemotherapy was much slower than reported by others.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00494-05 LPD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Analysis of T Cell Responses in Human Leishmaniasis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	D. Sacks	Senior Investigator LPD, NIAID
Others:	A. Cooper C. Karp F. Neva	Fogarty Fellow Guest Researcher Chief LPD, NIAID LPD, NIAID
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Cell Biology and Immunology Section		
INSTITUTE AND LOCATION NIH, NIAID, Bethesda, Maryland		
TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 2.8	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <p>Immunity to infection by the intracellular parasite <i>Leishmania</i> is mediated by sensitized T cells; however, the antigens which they recognize are still poorly defined. We have previously shown that the cell-mediated response of individuals with healed or healing forms of cutaneous leishmaniasis occurs to as many as 50-70 distinct antigens; however, it is unclear whether all the antigens capable of inducing a T cell response are important in protective immunity. As this question can only be addressed using purified distinct antigens directly in vaccine studies, and as these are most conveniently obtained through molecular cloning, we have begun to examine the T-cell immunogenicity of recombinant leishmanial antigens initially selected on the basis of their reactivity with human kala-azar serum. Although this approach relies upon overlap of T- and B-cell epitopes, a significant proportion of recombinants so selected showed immunogenicity in initial human T-cell proliferation assays. Work is presently underway to purify these putative T-cell antigens for further characterization. Investigation of the T cell response of leishmania patients to soluble antigen pulsed monocytes or monocytes infected with amastigotes has demonstrated the fundamental role of the CD4 subset of T cells in mounting both a proliferative and gamma interferon response to both sources of antigen. The role of CD8 cells in these responses varies between patients and the pattern of CD8 mediated response does not correlate with the source of antigen, i.e., endogenous (infected monocyte) or exogenous (antigen pulsed monocyte). Although no correlation was found between CD8 responses and live antigen, the use of live antigen to prestimulate patient cells has illustrated an enhanced gamma interferon response by mucocutaneous patient T cells when compared to cells from cutaneous patients.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AI 00512-04 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Definition Of Filarial Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. B. Nutman Senior Investigator

Others: N. Raghavan Visiting Scientist
E. Lobos Visiting Scientist
A. D. Klion Medical Staff Fellow
E. A. Ottesen Senior Investigator

COOPERATING UNITS (if any)

New England Biolabs, Beverly, Mass (F. Perler, L. McReynolds, C. Maina), Department of Geographic Medicine, University of Alabama Birmingham (D. Freedman)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.9

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

In the study of the human filariases, progress has been hampered by 1) the lack of defined parasite antigens; 2) the broad immunological cross-reactivity seen among the eight filarial species of humans; and 3) the dearth of abundant parasite material. The objectives of this project are to define and generate filarial proteins that are important in inducing parasite-specific immune responses in the human host and to understand, at a molecular level, the differences among related filarial species.

cDNA and genomic libraries have been either constructed (*Brugia malayi*, *Loa loa* and *Wuchereria bancrofti*, *Onchocerca volvulus*) or made available (*Onchocerca volvulus*, *Brugia malayi* larval cDNA library) so that screening with defined patient sera, patient T cells or parasite DNA could be performed. Recombinant antigens and probes have been identified that a) encode immunoreactive and potentially protective molecules of *W. bancrofti*; b) can distinguish among related filarial species by restriction fragment length polymorphisms, PCR or direct Southern blotting; c) identify repeated segments of either the *W. bancrofti* genome or that of *Loa loa*; d) may be of potential diagnostic importance; and e) induce immediate hypersensitivity type responses in lymphatic filarial syndromes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00579-02 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Studies on Immune Regulation in Toxoplasmosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	A. Sher	Senior Investigator	LPD/NIAID
Others:	R. Gazzinelli	IRTA Fellow	LPD/NIAID
	E. Denkers	IRTA Fellow	LPD/NIAID
	F. Hakim	Staff Fellow	I/NCI
	G.M. Shearer	Senior Investigator	I/NCI
	H. Morse	Chief	LIP/LPD
	A.W. Cheever	Asst. Chief	LPD/NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 10892

TOTAL MAN-YEARS

2.5

PROFESSIONAL

2.0

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

The overall aim of this project is to analyze the immune response to *Toxoplasma gondii* in order to define which cellular immune components and parasite target antigens are involved in the control of infection and its breakdown in immunocompromised hosts.

Progress was made this year in the following areas:

A. Demonstration of CD8+ cytolytic activity against *T. gondii* infected cells. CD8+ cells from vaccinated mice were shown to lyse infected or antigen pulsed macrophages *in vitro*. An antigenic fraction was partially purified which contains the appropriate target antigens.

B. Investigation of T cell subset requirements for reactivation. Reactivation of *T. gondii* in chronically infected mice was shown to be due primarily to a loss in CD8+ T cell function.

C. Establishment of a MAIDS - toxoplasmosis model. Infection of mice with a retrovirus mixture (MAIDS) was shown to reduce susceptibility to challenge infection and cause partial reactivation of latent infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00244-09 LPD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Developmental Adaptation of *Trypanosoma cruzi* to the Vertebrate Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. Sher Section Head LPD, NIAID

Others: S. Heath Visiting Fellow LPD, NIAID

COOPERATING UNITS (If any)

Yale University (K. Joiner, G. Ogden)

University of Sao Paulo, Brazil (T. L. Kipnis, D. V. Tambourgi)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.0

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

Terminated

LABORATORY OF VIRAL DISEASES
1990 ANNUAL REPORT
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LABORATORY OF VIRAL DISEASES
ANNUAL REPORT, 1991
SUMMARY

The Laboratory of Viral Diseases carries out investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. These studies are designed to increase fundamental knowledge as well as to facilitate the development of new approaches to the prevention and treatment of virus infections. Current topics of basic research include virus entry into cells, regulation of gene expression, mechanisms of DNA replication, assembly and transport of viral proteins and particles, action of virus growth factors, determinants of virus virulence, host resistance genes, and viral targets of humoral and cellular immunity. Applied areas of investigation include the development of recombinant expression vectors, candidate vaccines, and antiviral agents. These studies involve a wide range of DNA and RNA viruses including human immunodeficiency virus.

REGULATION OF VIRUS GENE EXPRESSION

Viruses provide advantageous systems for studying basic aspects of gene expression and can be used as expression vectors. Several different virus systems are currently under investigation by members of LVD.

POXVIRUSES. Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. In order to express and replicate their genomes outside of the nucleus, poxviruses must encode enzymes for these processes. This feature makes poxviruses, among which vaccinia virus is the prototype, unique for studying the regulation of transcription. Additional information regarding transcription also is needed to improve vaccinia virus expression vectors, which are currently being evaluated as live vaccines against several diseases including AIDS. During the past year, progress has been made in identifying additional viral genes that are RNA polymerase subunits and transcription factors. Further evidence was obtained for a cascade mechanism of gene regulation.

Vaccinia virus encoded RNA polymerase. The DNA-dependent RNA polymerase of vaccinia virus contains 8 to 10 polypeptides. Previous studies from this laboratory indicated that the RNA polymerase subunits are virus-encoded and the genes for one large and two small subunits were identified. Three additional RNA polymerase subunit genes encoding polypeptides of 132,000, 35,000, and 30,000 Daltons were identified within the vaccinia virus genome and sequenced. All three genes are expressed both early and late in infection and the protein subunits are assembled into the RNA polymerase complex that is packaged in virions. The 132,000 Dalton subunit is homologous to the second largest subunits of RNA polymerases of prokaryotes and eukaryotes. The 30,000-Dalton subunit is homologous to a eukaryotic transcription elongation factor. All of the subunits are expressed throughout the cycle of infection.

(Ahn, Amegadzie and Moss)

Vaccinia virus early transcription factor. The early transcription factor (VETF) is a heterodimer consisting of virus-encoded 70-kD and 82-kD subunits. Evidence was obtained that VETF binds to specific nucleotides preceding and following the transcription initiation site and in so doing bends the DNA.

(Broyles and Moss)

A transcription factor for expression of vaccinia virus late genes is encoded by an intermediate gene. A factor, designated VLTF-1, that is required in vitro for specific transcription of vaccinia virus late genes was previously isolated from vaccinia virus-infected cells. Our subsequent genetic experiments identified three vaccinia virus genes, encoding proteins of 17, 26, and 30 kDa, that together *trans* activate late gene expression in vivo. Present data indicate that the 30 kDa protein comprises part, if not all, of VLTF-1 activity.

(Keck, Winters and Moss)

Parvoviruses. Parvoviruses are small viruses that contains a single-stranded DNA genome. One well studied member of this family, adeno-associated virus (AAV), is dependent for replication on coinfection with an adenovirus or herpesvirus. AAV gene expression appears to depend on cellular and helper viral gene products and is regulated by post-transcriptional mechanisms that also may be important for eukaryotes.

Regulation of synthesis of AAV structural proteins at the translational level. Infectious AAV plasmids containing site-directed mutations were used to confirm that structural protein B is initiated at ACG, a previously unknown eukaryotic translation initiation codon. Furthermore, mutations of this codon affected the levels of structural protein B as well as that of structural protein C, both of which are expressed from the same transcript. Thus, the coordinate expression of B and C depends on the relatively weak ACG initiation codon of B.

(Sebring, Wong, Muralidhar and Rose)

Regulation of synthesis of AAV structural proteins by alternative splicing. The only functional mRNA for expression of structural protein A is generated by alternative splicing. A mutation in the splice acceptor site of the A message abolishes the synthesis of B and C while allowing synthesis of A.

(Sebring, Wong, Muralidhar and Rose)

VIRUS DNA REPLICATION

Viruses are useful systems for analyzing the diversity of mechanisms employed in DNA replication. In addition, the virus encoded factors provide potential targets for chemotherapy.

Poxviruses. Poxviruses provide a unique experimental system, since the enzymes and factors are encoded within the virus genome and replication occurs in the cytoplasm. Previous studies revealed that vaccinia virus DNA is replicated as concatemeric structures which are resolved into unit length genomes with hairpin ends.

Resolution of vaccinia virus DNA concatemer junctions is sequence-specific. An analysis of a series of symmetrical site directed mutations revealed that the nucleotide sequence necessary for resolution of vaccinia virus DNA concatemer junctions is (A/T)TTT(A/G)N_{7,9}AAAAAA, in which N can be varied. This nucleotide sequence is highly conserved among poxviruses and is found proximal to the hairpin loop of members of the leporipox, avipox, and capripox families.

(Merchlinsky)

Conserved nucleotides are essential for DNA polymerase function. Procedures were developed for the efficient use of the polymerase chain reaction to introduce alterations into codons for conserved residues within the vaccinia virus DNA polymerase. The data revealed that several non-conservative substitutions were not compatible with the isolation of viable mutant viruses.

(DeFilippes)

HERPES SIMPLEX VIRUS (HSV). HSV and other members of the herpesvirus family are significant human pathogens. The study of HSV DNA synthesis is useful as a model for eukaryotic DNA replication and for designing new anti-viral strategies. Previous work from this laboratory demonstrated that seven HSV genes are necessary and sufficient for authentic origin-dependent DNA replication. Current efforts are directed toward studying this process with purified proteins.

UL42 increases the processivity of the DNA polymerase. The HSV DNA polymerase purified from infected HeLa cells consists of a stable complex of two polypeptides: UL30, the catalytic subunit, and UL42, an accessory subunit. Several lines of evidence support the view that UL42 increases the efficiency of the DNA polymerase by increasing its processivity. Results obtained using a novel synthetic model primer-template, suggest that UL42 increases the processivity of DNA polymerase by acting as a sliding clamp, reducing the probability that the polymerase will dissociate from the elongating DNA chain after each cycle of catalysis.

(Gottlieb and Challberg)

UL5 and UL52 catalyze helicase and primase activities. The UL5, UL8, and UL52 polypeptides form a three protein complex that has both helicase and primase activities. Purification of the isolated subunits and subcomplex associations of subunits suggests that UL5 and UL52 act together to catalyze both helicase and primase activities. On the basis of sequence motifs, UL5 may have the active site for helicase function, but UL5 is not active as a helicase in the absence of UL52, which may have the active site for primase.

(Sherman, Klinedinst, Gottlieb and Challberg)

UL8 is required for lagging strand DNA synthesis. Although UL8 is required for neither helicase nor primase activities, it is required for efficient utilization of primers by DNA polymerase in a model system for lagging strand synthesis. UL8 appears to stabilize the association of nascent primers with the template, increasing the probability that primers are elongated by DNA polymerase.

(Sherman, Gottlieb and Challberg)

Helicase activity of UL9 is stimulated by ICP8. UL9 binds specifically to origins of DNA replication. In the absence of ICP8, the UL9 helicase will unwind DNA strands up to about 100 base pairs in length; in the presence of ICP8, DNA strands greater than 1-2000 bp are efficiently unwound. Since no heterologous single-stranded DNA binding protein can substitute for ICP8, these data are consistent with the idea that there is a specific interaction between UL9 and ICP8.

(Fierer and Challberg).

ADENO-ASSOCIATED VIRUS. The AAV genome is a linear single-stranded DNA molecule with partially palindromic inverted terminal repeats. Replication of AAV DNA occurs by a mechanism involving self-primed synthesis.

Synthesis of concatemeric AAV DNA without AAV-specified proteins. Following coinfection of restrictive monkey cells with AAV2 and human adenovirus 5, NS and structural AAV protein synthesis was inhibited > 98% and AAV DNA synthesis occurred but instead of normal unit length duplex and single stranded molecules, long concatemers accumulated. This restriction of AAV replication was completely reversed by preinfection with SV40 or by using a simian adenovirus as a helper. Taken together, these results suggest that synthesis of AAV concatemeric DNA may not require AAV nonstructural proteins, but that one or more of these proteins is required to resolve AAV concatemeric intermediates to unit length duplex DNA molecules, presumably by nicking at a specific site(s).

(Sebring, Wong, Muralidhar and Rose)

HSV genes enhance AAV DNA synthesis. AAV DNA synthesis was efficiently induced by cotransfections of vero cells with pAV1 and at least five of the seven HSV-required genes plus ICP4. In contrast to their requirement for HSV DNA synthesis, none of the five HSV-required genes was absolutely necessary for AAV DNA replication. Furthermore, they appeared to exert a cumulative effect on the stimulation of AAV DNA synthesis. This suggests that products of these genes may be enhancing AAV DNA synthesis by participating in a DNA replication complex that is partially composed of cellular DNA synthesizing factors.

(Mishra and Rose)

SYNTHESIS, ASSEMBLY, AND TRANSPORT OF VIRAL PROTEINS AND PARTICLES

Information regarding the folding, assembly, and transport of viral glycoproteins and the formation of virus particles is of intrinsic interest and practical importance, since it determines the antigenic structure recognized by neutralizing antibody.

INFLUENZA VIRUS. The trimeric influenza hemagglutinin (HA) molecule is the best characterized viral envelope protein and therefore serves as an excellent model. Additionally, influenza virus remains a significant human pathogen, and the HA is a critical antigen for protecting individuals against disease.

Site of oligomerization of the HA. Further cytochemical studies using monoclonal antibodies specific for monomeric and trimeric forms of HA suggested that oligomerization occurs in the intermediate compartment that exists between the endoplasmic reticulum and Golgi complex. Analysis of mutant forms of HA are also consistent with this interpretation.

(Eisenlohr, Rus, Bennink and Yewdell)

Action of brefeldin A. Brefeldin A, a fungal metabolite that blocks exocytosis of secretory and integral membrane proteins, was coupled to a fluorescent compound and its subcellular distribution was determined. These studies demonstrated that the compound has a particular affinity for the endoplasmic reticulum.

(Bennink and Yewdell)

HUMAN IMMUNODEFICIENCY VIRUS (HIV). HIV is the etiological agent of acquired immune deficiency syndrome (AIDS). At present there is not effective vaccine against this virus and therapeutic agents provide only limited help. The envelope protein is the best target for neutralizing antibody and is therefore of particular importance for development of a vaccine.

Assembly of the HIV envelope protein. A detailed analysis of the biosynthetic events undergone by the HIV-1 envelope protein expressed by a recombinant vaccinia virus was performed. Early folding events that occurred in the endoplasmic reticulum included disulfide bond formation ($t_{1/2} \approx 10$ min), folding of envelope protein into a form competent to bind CD4 ($t_{1/2} \approx 15$ min), and specific and transient association and dissociation with GRP78-BiP ($t_{1/2} \approx 30$ min). Studies with brefeldin A suggested that assembly occurred in the endoplasmic reticulum while cleavage of gp160 into gp120/gp41 subunits occurred in a post-endoplasmic reticulum compartment. Transport to the Golgi was monitored by modification of N-linked sugars to forms partially resistant to endoglycosidase H. The kinetics of endoglycosidase H resistance were nearly identical to the kinetics of gp160 cleavage ($t_{1/2} \approx 80$ min).

(Earl, Doms and Moss)

Effects of C-terminal and internal deletions on the synthesis, transport, biological properties, and antigenicity of the HIV-1 envelope protein. A family of recombinant vaccinia viruses that express N-terminal overlapping *env* proteins was constructed. The 747- and 851-aminoacid proteins were cleaved, were expressed on the surface of infected cells, and bound CD4. The 635-aminoacid *env* protein was cleaved inefficiently, and both the precursor and product were secreted, indicating absence of the transmembrane sequence. The 635- as well as the 502-aminoacid protein, which was also largely secreted, could still bind CD4. When amino acids at the gp120-gp41 junction were deleted, proteolytic cleavage of gp160 did not occur. Nevertheless, gp160 was inserted into the plasma membrane and bound soluble CD4. The predominant conserved B-cell epitopes were mapped to gp41 and the C terminus of gp120, whereas cytotoxic T-cell epitopes were distributed throughout the length of the glycoproteins.

(Earl, Doms and Moss)

Structure/function, studies of the CD4/HIV envelope glycoprotein interaction. HIV infection of a human T-lymphocyte is initiated by specific binding of the external subunit (gp120) of the virus envelope glycoprotein to CD4 molecules on the surface of the target cell; the binding event is then followed by direct fusion between the viral and cell membranes. The molecular basis of the fusion event may involve structural changes in the envelope glycoprotein leading to exposure of the hydrophobic N-terminus of the transmembrane subunit (gp41). In this context, investigations of CD4/HIV envelope interactions revealed that binding of a soluble form of CD4 stimulated release of gp120 from gp120/gp41 expressed at the cell surface. A similar gp120 release phenomenon was induced by peptide derivatives from the CDR3 region of CD4 (provided by Lee Eiden, NIMH) that also inhibited fusion mediated by the CD4/envelope glycoprotein interaction. Moreover, a peptide derivative with a specific single amino acid substitution had substantially diminished activity for both inhibition of fusion and gp120 release.

(Broder and Berger)

VACCINIA VIRUS. There are two types of infectious vaccinia virus particles: intracellular naked virions (INV) and extracellular enveloped virions (EEV). The latter form of the virus is thought to be important for virus spread and virulence.

Formation of extracellular vaccinia virus and cell-to-cell virus transmission are prevented by deletion of the gene encoding the 37,000 Da outer envelope protein. To determine the biological role of the enveloped form of vaccinia virus, we produced and characterized a mutant that is defective in EEV formation. The strategy involved deletion of the gene encoding a 37,000 Da protein (VP37) that is specific for the outer envelope of EEV. The desired mutant was defective in production of plaques and extracellular virus but made normal amounts of INV. Electron microscopic examination indicated that the mutant virus particles, unlike wild-type, were neither wrapped with Golgi-derived membranes nor associated with the cell surface. These results indicate that VP37 is required for EEV formation and also plays a critical role in the local cell-to-cell spread of virus.

(Blasco and Moss)

Profilin homolog encoded by vaccinia virus is not essential for replication. A gene with homology to profilin, an actin-binding protein, was found to be encoded by vaccinia virus. Deletion of this gene had no effect on vaccinia virus-induced changes in actin fibers or actin-associated event including intracellular virus movement and release of mature virions.

(Blasco, Cole and Moss)

Development of inducer-dependent conditional-lethal mutant animal viruses to study virion assembly. Regulatory elements of the *Escherichia coli lac* operon were used to construct an inducer-dependent conditional-lethal mutant animal virus. The gene encoding the repressor protein of the *lac* operon was integrated into the vaccinia virus genome so that it was expressed constitutively, and the *lac* operator was inserted next to the promoter of a gene that encodes an 11-kDa virion-associated protein. The addition of inducer provided permissive conditions for isolation of a conditional-lethal mutant virus. Under nonpermissive conditions, the isolated virus did not form plaques, and the yield was decreased by at least 1000-fold under one-step growth conditions. Transcription of the operator-controlled gene was inducer-dependent and necessary for synthesis of the 11-kDa structural protein.

(Zhang and Moss)

VIRUS-HOST INTERACTIONS

Recent studies have revealed that some viruses encode proteins to defend themselves from the specific and non-specific immune responses of the host. This is especially apparent with poxviruses which encode many proteins that have no demonstrable roles during replication in cultured cells yet are important for virulence in animals. Investigations of the mechanisms of virus pathogenesis provide information about the host immune mechanisms and are important for the construction of safe, effective recombinant vaccines.

Cowpox anti-inflammatory protein. Previous studies demonstrated that a virus-encoded 38-kDa protein is important in inhibiting the host inflammatory response to cowpox infection of chicken embryos. Evidence has been obtained that the lipoxygenase pathway of arachidonic acid metabolism is the target of the 38-kDa protein. Cells infected with wild-type virus, but not with mutants defective in 38-kDa protein synthesis, produce 14,15-diHETE which may be the chemoattractant for the inflammatory response.
(Palumbo and Buller)

Role of interferons in host response to viral infection. Investigations of ectromelia virus infection of mice via the foot-pad route revealed that gamma interferon production by the host is relatively more important than alpha and beta interferons for recovery from disease.
(Karupiah and Buller)

Inhibition of complement enhanced virus neutralization by the major secretory protein of vaccinia virus. Previous studies demonstrated that a vaccinia virus-encoded 35-kDa homolog of eukaryotic complement control proteins was secreted from infected cells and could inhibit complement mediated hemolysis in vitro. Further studies indicate that this protein also can prevent complement enhanced neutralization of vaccinia virus by antibody. In addition, skin lesions in rabbits formed by a vaccinia virus mutant with the complement control gene deleted are smaller and heal more rapidly than those of wild-type virus suggesting that the viral protein plays a significant role in defense from the host immune system.

(Isaacs and Moss)

VIRAL IMMUNOLOGY

Class I molecules of the major histocompatibility complex class (MHC) consist of a highly polymorphic heavy chain complexed to B₂macroglobulin. Class I molecules are expressed on virtually all cell types. Their sole function is to bind antigens and present them to T cell bearing CD8 molecules. These T cells are known as cytotoxic T lymphocytes (CTLs) due to their ability to lyse histocompatible cells in an antigen specific manner. CTLs play a critical role in eradicating intracellular pathogens and tumors. On the negative side, they are involved in organ rejection, and in many autoimmune dyscrasia.

Association of peptides with MHC class I molecules. Class I molecules bind peptides of between 8 and 10 residues. Since the processing pathway begins in the cytosol and association with class I molecules occurs in an exocytic compartment, proteolysis could potentially occur in either (or both) compartment. To define the requirements for transport of determinants from the cytosol to class I molecules in an exocytic compartment, the

capacity of cells to present small peptides biosynthesized in the cytosol was examined. A recombinant vaccinia virus expressing a 9 residue peptide sensitized target cells for lysis as efficiently as a recombinant expressing in full length protein antigen. Thus, cells are capable of transporting peptides of 9 or 10 residues from the cytosol to class I molecules. Further studies showed that amino acid residues flanking the nonapeptide are critical in determinant selection.

(Eisenlohr, Yewdell and Bennink)

Cells deficient in antigen presentation. A number of mutant cell lines have been identified that are deficient in the presentation of endogenously synthesized protein antigens but not peptides added exogenously to cells. Although RMA/S cells have been reported to be completely deficient in processing influenza viral proteins for CTL recognition, they are also relatively resistant to infection with influenza. Vesicular stomatitis virus (VSV) grows well in RMA/S cells and VSV antigens are presented. Nevertheless, RMA/S cells required more antigen and assembled or transported the class I peptide complex more slowly than non-mutant cells. This is consistent with a partial deficit in a unique cellular function required for presentation and processing or a complete deficit and the operation of a less efficient alternative pathway.

(Bennink and Yewdell)

VIRUS EXPRESSION VECTORS

Use of expression vectors has become an important part of recombinant DNA technology. Vaccinia virus is now widely used for expression of proteins in mammalian cells and has proven particularly useful for determining the targets of humoral and cellular immunity. Recombinant vaccinia virus is currently being tested as a candidate AIDS vaccine in humans and a rabies vaccine in wild-life. The unique DNA integration and other properties of some parvoviruses suggest that they might be of particular use for gene therapy (see section on antiviral agents).

Development of an inducible system to regulate expression of the bacteriophage T7 RNA polymerase gene in a vaccinia virus vector. Recently, we described a chimeric system capable of attaining high expression of target genes. This system is based on confection of cultured cells with two recombinant vaccinia viruses. One recombinant virus provides constitutive expression of bacteriophage T7 RNA polymerase, which drives expression in the second virus of a T7-promoter-controlled target gene. Transfer of the *E. coli lac* operator/repressor system to regulate the expression of T7 RNA polymerase in vaccinia virus permitted stable incorporation of a T7-promoter-controlled reported gene in the same viral genome. This single virus system, referred to as the Vac/Op/T7 system, expressed the model protein β -Gal at a level higher than that achieved using the Vac/T7 coinfection system. The Vac/Op/T7 system may be more economical, easier to use, and less subject to variation than a coinfection system for large-scale protein production.

(Fuerst and Moss)

Cytoplasmic expression system based on constitutive synthesis of bacteriophage T7 RNA polymerase in mammalian cells. A mouse cell line that constitutively synthesizes the bacteriophage T7 RNA polymerase was constructed. The *in vivo* activity of the bacteriophage polymerase was demonstrated by transfection of a plasmid containing the chloramphenicol acetyltransferase (CAT) gene flanked by T7 promoter and termination signals. Synthesis of CAT was dependent on the presence of a cDNA copy of the untranslated region of encephalomyocarditis virus (EMCV) RNA downstream of the T7 promoter, consistent with the absence of RNA-capping activity in the cytoplasm. In addition, expression of CAT from pT7EMCAT was increased more than 500-fold when the transfected cells also were infected with wild-type vaccinia virus. A protocol for high-level expression involved the infection of the T7 RNA polymerase cell line with a single recombinant vaccinia virus containing the target gene regulated by a T7 promoter and EMCV untranslated region.

(Elroy-Stein and Moss)

Reverse guanine phosphoribosyltransferase selection of recombinant vaccinia viruses. A procedure was developed for the selection of recombinant vaccinia viruses with applicability to poxvirus mutagenesis studies and to the use of vaccinia virus as an expression vector. The method depends on the specific inability of a recombinant vaccinia virus expressing the *Escherichia coli* guanine phosphoribosyltransferase gene (*gpt*) to form plaques on a hypoxanthine—guanine phosphoribosyltransferase-negative line of mouse fibroblasts in the presence of 6-thioguanine. Recombinant viruses that have the *gpt* removed can form plaques under selection conditions, thus providing a simple and efficient selection protocol.

(Zhang and Moss)

CHARACTERIZATION OF NEWLY DISCOVERED VIRUSES

HUMAN HERPESVIRUSES. Until recently, only 5 human herpesviruses were known. A few years ago, human herpesvirus 6 (HHV-6) was discovered and this was followed by the isolation of human herpesvirus 7 (HHV-7) in this laboratory. Both of these new human herpesviruses are lymphotropic and latent infections are very common. HHV-6 has been associated with roseola, a disease of infants and may cause rare encephalitic complications. In addition reaction of HHV-6 may occur in patients undergoing immunosuppression.

Structure of HHV-6 and HHV-7 genomes. Large direct repeats of 12 to 13 kilobases were found to flank the central region of the HHV-6 genome. These sequences are responsible in part for strain to strain variations. A large portion of the genome of HHV-7 has been cloned and the homology of each to HHV-6 was determined. PCR-specific primers for HHV-6 and HHV-7 have been developed.

(Danovich, Wyatt and Frenkel)

Virion morphogenesis. Nuclear concatemeric genomes are cleaved prior to exit of viral DNA into the cytoplasm. The egress of viral structural particles into the cytoplasm and the envelopment of tegumented virions has been studied, and EM analyses revealed the existence of an intranuclear compartment where the virion tegumentation occurs.

(Roffman, Avidor and Frenkel)

Requirements for HHV-6 replication. T cell activation is required for HHV-6 replication. Differences in the efficiency of activation occurs with PHA, anti-CD3⁺ CD28 anti CD3⁺ IL2, PMA and ionomycin. In the thymocyte, activation can occur by IL-2 dependent triggering via the T cell receptor.

(Avidor, Roffman and Frenkel)

Molecular epidemiology of human herpesviruses 6 and 7. Examination of sera of healthy adults and children has shown that HHV-6 and HHV-7 are highly prevalent viruses which infect in early childhood. Seroconversion for HHV-7 occurs at a later age than seroconversion for HHV-6. Examination of virus isolates obtained from roseola patients as well as by reactivation of peripheral blood lymphocytes of healthy individuals, revealed that HHV-6 strains are highly conserved viruses, but they fall into two distinct groups of viruses which differ in their growth properties, restriction enzyme patterns, antigenic reactivity and disease association. Only one of these virus groups (prototype Z29 strain) appears to be associated with roseola infantum. It is as yet unknown whether viruses of the second virus prototype U1102 strain infect in early childhood or are associated with any disease. In additional studies regarding disease association of HHV-6 and HHV-7, no evidence was found for involvement of HHV-6/7 in Kawasaki Disease. Moreover, there was no compelling evidence for the involvement of HHV-6 or HHV-7 in chronic fatigue syndrome.

(Wyatt and Frenkel)

DEVELOPMENT OF ANTIVIRAL AGENTS

New molecular approaches are being developed for the prevention and treatment of viral diseases. These include recombinant subunit and live vaccines, targeted therapeutics, and novel ways of providing intracellular immunity.

gp160 - candidate HIV-1 vaccine. A subunit vaccine composed of HIV-1 envelope precursor gp160 has recently been approved for phase 1 clinical testing. The vaccine was produced under a CRADA agreement with IMMUNO using recombinant expression vectors made in the Laboratory of Viral Diseases. A recombinant vaccinia virus that expresses gp160 at even higher levels has been made and will facilitate increased production.

(Chakrabarti, Earl and Moss)

CD4-PE - candidate HIV therapeutic. A genetically engineered hybrid toxin CD4-PE40, composed of the HIV envelope protein-binding domain of CD4 and the translocation and ADP ribosylation domains of Pseudomonas exotoxin A, was produced as a collaborative project between NIAID and NCI, and has been licensed by the Upjohn pharmaceutical company. Phase I clinical testing is planned for this year. Additional laboratory testing indicated that the hybrid toxin is active against cells expressing diverse forms of HIV and SIV envelope glycoprotein, including those with weak binding affinity for CD4. New forms of CD4-PE have been shown to enhance killing of HIV-infected cells.

(Berger and Moss)

Parvovirus (AAV) vectors provide intracellular resistance to HIV-1. Stable resistance to HIV-1 was induced in human lymphocyte cell lines using a recombinant AAV-based vector encoding an antisense RNA complementary to the HIV-1 TAR sequence, which is common to all HIV transcripts and critical for efficient virus transcription and replication. Cell clones that constitutively expressed this antisense RNA, specifically inhibited (>90%) HIV LTR-directed gene expression, HIV replication, and production of infectious HIV particles.
(Chatterjee, Wong, Rose and Johnson)

Parvovirus (AAV) vectors provide intracellular resistance to HIV-1. Stable resistance to HIV-1 was induced in human lymphocyte cell lines using a recombinant AAV-based vector encoding an antisense RNA complementary to the HIV-1 TAR sequence, which is common to all HIV transcripts and critical for efficient virus transcription and replication. Cell clones that constitutively expressed this antisense RNA, specifically inhibited (>90%) HIV LTR-directed gene expression, HIV replication, and production of infectious HIV particles.
(Chatterjee, Wong, Rose and Johnson)

Parvovirus (AAV) vectors provide intracellular resistance to herpes simplex virus (HSV). An AAV-based antisense vector targeting the early HSV-encoded transactivator, ICP4, was used to transduce stable intracellular resistance to HSV-1 as well as HSV-2. A 1,000 to 10,000 fold inhibition of HSV production, correlated with decreased ICP4 synthesis, was observed.

(Wong, Rose, Chatterjee)

Intracellular antibody-mediated influenza virus neutralization. Electroporation of a monoclonal antibody to the nucleoprotein of influenza virus was able to block replication of both H1N1 and H3N2 human influenza virus strains. Efforts to develop cell lines expressing this antibody is in progress.

(Ffrench, Bennink and Yewdell)

ADMINISTRATIVE CHANGES

During the past year, the Viral Immunology and Molecular Genetics Sections moved from the Rockville Twinbrook facility to Building 4 on the Bethesda campus. Only the Molecular Structure Section now remains at Twinbrook. This change resulted in the consolidation of four of the five sections of LVD and should considerably improve scientific interactions.

Honors, awards, and service. Members of LVD received numerous invitations to give major lectures and seminars in the United States as well as in foreign countries. Drs. Bennink, Challberg, Moss and Rose serve on the editorial boards of one or more scientific journals. Dr. Challberg is an advisor to the National Science Foundation; Dr. Moss is a member of the National Academy of Sciences, and this year received the Invitrogen Award for research on Eukaryotic Gene Expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00123-25 LVD												
PERIOD COVERED October 1, 1990 to September 30, 1991														
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Structure and Replication of Poxvirus DNA														
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">M. Merchlinshy</td> <td style="width: 20%;">Expert</td> <td style="width: 30%;">LVD, NIAID</td> </tr> <tr> <td>Others:</td> <td>B. Moss</td> <td>Chief</td> <td>LVD, NIAID</td> </tr> <tr> <td></td> <td>A. Levy</td> <td>Visiting Fellow</td> <td>LVD, NIAID</td> </tr> </table>			PI:	M. Merchlinshy	Expert	LVD, NIAID	Others:	B. Moss	Chief	LVD, NIAID		A. Levy	Visiting Fellow	LVD, NIAID
PI:	M. Merchlinshy	Expert	LVD, NIAID											
Others:	B. Moss	Chief	LVD, NIAID											
	A. Levy	Visiting Fellow	LVD, NIAID											
COOPERATING UNITS <i>(if any)</i> None														
LAB/BRANCH Laboratory of Viral Diseases														
SECTION Genetic Engineering Section														
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892														
TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews					
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<input type="checkbox"/> (a1) Minors														
<input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>Poxviruses provide a unique system for studying the synthesis of DNA. Required enzymes and factors are encoded within the viral genome and DNA synthesis and processing occurs within the cytoplasmic compartment of the cell. Therefore, it has been possible to apply genetic and biochemical approaches to the study of DNA replication. My effort has been towards ascertaining the structure and mode of replication of the poxvirus genome with particular emphasis placed on understanding the processing of the telomere-like hairpin structure and the enzymes involved in its replication. The replication of vaccinia virus proceeds through concatemeric intermediates which are resolved into unit length DNA. Plasmids containing the telomere replicative intermediate were, after transfection into cells infected with vaccinia virus, replicated and resolved into linear minichromosomes with sealed terminal hairpins, providing a system to study the <i>cis</i> acting DNA sequences required for telomere resolution. Mutational analysis has demonstrated that aDNA sequence, highly conserved among poxviruses, as well as the palindromic structure of the concatemer junction, is essential for resolution, and that resolution occurred by conservative strand exchange. A model for resolution involving site-specific recombination and orientated branch migration is consistent with this data. Gene products with activities consistent with processing of replicative intermediates have been identified and isolated as first steps in the biochemical characterization of DNA replication in poxviruses. A procedure has been developed for the generation of viral genomes containing large inserts of foreign DNA by ligation of subgenomic viral DNA fragments in the presence of the insert DNA. These DNA molecules are transfected into cells where they can be packaged into viral particles and subsequently propagated as virus.</p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00126-18 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analyses of Vaccinia Virus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frank M. DeFilippes Research Physicist LVD, NIAID

Others: None

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The experiments are designed to test the assumption that the conserved residues (CR) of a protein are essential for its function. Procedures were developed for the efficient use of the polymerase chain reaction (PCR) to introduce alterations into codons for the CRs of vaccinia virus DNA polymerase. These alterations are linked to a mutation which confers resistance to aphidicolin (AP). A single priming oligonucleotide with an alteration of a codon for a specific CR and the mutation which confers AP resistance (APr) was used in the PCR to produce an altered DNA which was introduced into virus infected cells by a marker transfer (MT) technique. An extract of these cells was diluted and altered virus was detected by plaque formation in AP medium. In one experiment, alteration of a CR which caused a "mild" substitution of a wild type amino acid was used to form a Bgl restriction site. Most of the APr plaques tested contained the altered sequence with the Bgl site. However, I found that "drastic" substitutions of highly conserved residues, such as substituting aspartic acid for tyrosine are, at best, rare events. In these experiments, the few APr plaques which developed did not contain the linked "drastic" substitution. Although this result indicates that such substitutions are not allowed, the MT reaction must be controlled to set the limits of the experiment. For this reason every priming oligonucleotide which contains an alteration of a CR also contains the alteration to introduce the Bgl site as well as the APr mutation. PCR methods have been developed to amplify small amounts of virus DNA from APr plaques to check the sequence of the DNA for the desired alterations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00294-10 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Structure and Function of Adenovirus DNA		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI:	J. A. Rose	Section Head LVD, NIAID
Others:	E. Sebring	Research Chemist LVD, NIAID
COOPERATING UNITS <i>(if any)</i> R. McPherson, M.D., Department of Pathology, Scripps Clinic, San Diego, CA		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.25	PROFESSIONAL: 0.75 OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided)</i> Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and function of certain sequences (e.g., the inverted terminal repeats) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the <u>VA RNA gene/transcript</u> and <u>inverted terminal repeats</u> in Ad DNA. We have continued to investigate the specific functions of several early Ad genes, e.g., the <u>VA and DNA-binding protein genes</u> . Our results indicate that these latter genes are involved in the <u>regulation of translation</u> of certain viral mRNAs. Among methods used are <u>gel electrophoresis</u> , <u>gene cloning</u> , <u>mutagenesis</u> , <u>base sequence analysis</u> , and <u>DNA transfection</u> .		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 A1 00295-10 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Helper Factors Required for Expression of the Adeno-Associated Virus Genome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J. A. Rose Section Head LVD, NIAID		
COOPERATING UNITS (if any) L. Mishra, Senior Staff Fellow, Division of Blood and Blood Products, CBER, FDA		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.2	PROFESSIONAL: 1.2	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) The main objectives of this project are (i) to identify the <u>helper virus genes that regulate expression of defective human parvovirus (AAV) genomes</u> , and (ii) to define their respective roles in AAV replication. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. Among methods used are <u>specific immunofluorescence</u> , <u>DNA cloning</u> , <u>gel electrophoresis</u> , <u>blot-hybridization</u> analyses, and <u>DNA transfection</u> of both simian and human cell lines.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00296-10 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Characterization and Production of Parvovirus Proteins		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI:	J. A. Rose	Section Head LVD, NIAID
Others:	E. Sebring	Research Chemist LVD, NIAID
	K. Wong	Senior Staff Fellow LVD, NIAID
	S. Muralidahar	Visiting Associate LVD, NIAID
COOPERATING UNITS <i>(if any)</i>		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.05	1.55	1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p> The main objectives of these studies are (1) to identify and characterize all <u>proteins</u> that are specified by the <u>defective human parvovirus</u> (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (2) to define the mechanism(s) by which the AAV proteins arise, and (3) to define specific functions of the AAV proteins. We have identified at least four AAV non-structural proteins. At least one of these proteins is necessary for viral DNA replication. <u>Post-translational processing</u> does not account for production of any AAV structural proteins although they share large segments of <u>sequences-in-common</u>. It is now clear, however, that these proteins originate from <u>independent in-frame initiations</u>. Mechanisms that regulate expression of AAV proteins are of fundamental interest, and we have shown that one AAV structural protein is initiated by a codon (ACG) not known previously to act as an <u>initiation codon</u> in higher eukaryotes. Furthermore, our current findings (i) support a "<u>scanning mechanism</u>" in the translational expression of polycistronic eukaryotic mRNAs, and (ii) demonstrate that <u>alternative mRNA splicing</u> is required for effective translational expression of the largest AAV capsid protein. Among methods used are <u>site-directed mutagenesis</u>, <u>affinity chromatography</u>, <u>gel electrophoresis</u>, <u>in vitro translation of viral RNA</u>, <u>DNA transfection</u>, <u>immunoprecipitation</u>, and <u>Western blotting</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00297-10 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. A. Rose Section Head LVD, NIAID

Others: E. Sebring Research Chemist LVD, NIAID

COOPERATING UNITS (if any)

S. Chattirjee, Assistant Professor, Dept. of Microbiology, Georgetown University

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The primary objective of this project is to define molecular and biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) DNA replication in both *in vivo* and *in vitro* systems. We have shown that AAV DNA synthesis can be initiated *in vitro* in cell-free extracts and that replicating forms that correspond to those identified *in vivo* can be synthesized with either endogenous or exogenously added templates. Recently, we have shown that AAV "rep" proteins may not be required for the synthesis of AAV concatemeric DNA intermediates, but that one or more of these proteins is required to resolve the long intermediates into unit length duplexes. The observed mode of AAV DNA replication may serve as a model for telomere replication in eukaryotes. Among methods used are affinity chromatography, gel electrophoresis, DNA sequence analysis, and restriction cleavage of DNA molecules and DNA transfection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00298-10 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Vaccinia Virus as an Expression Vector		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Moss	Chief LVD, NIAID
Others:	O. Elroy-Stein S. Isaacs	Special Volunteer Medical Staff Fellow LVD, NIAID LVD, NIAID
COOPERATING UNITS (if any) D. Kaslow, LPD, NIAID; T. Fuerst, MedImmune; H. Lester, California Institute of Technology; E. Wimmer, SUNY; J. Hay, USUHS; A. Davison, University of Glasgow.		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Genetic Engineering Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.2	PROFESSIONAL: 0.7 OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>Vaccinia virus has been developed into a novel eukaryotic expression vector with many uses. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is then used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. In a novel modification of the system, the bacteriophage T7 RNA polymerase gene was integrated into the vaccinia virus genome under control of a vaccinia promoter. The highly efficient and specific bacteriophage polymerase was then used to transcribe genes placed next to T7 promoters. In a further modification, the <i>Escherichia coli lac</i> operator system was used to regulate expression of the bacteriophage T7 RNA polymerase gene within the vaccinia virus genome. By doing this, it was possible for the first time to incorporate both the T7 RNA polymerase gene and a foreign gene under T7 promoter control in the same viral genome without compromising the viability of the virus. Expression of the foreign gene was obtained on addition of inducer. Still another modification of the system involved the formation of a cell line that constitutively express the bacteriophage T7 RNA polymerase thus eliminating the need to incorporate this gene into vaccinia virus. Several enhanced vectors were made for high expression with either the standard recombinant vaccinia system or the hybrid bacteriophage T7/vaccinia system. A new method of isolating recombinant vaccinia viruses, called reverse guanine phosphoribosyltransferase selection was developed. In collaborative studies, recombinant vaccinia viruses were used for expression of ion channels and receptors in frog oocytes, a candidate transmission blocking malaria vaccine, the poliovirus receptor, and varicella zoster envelope glycoproteins.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00306-10 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Orthopoxvirus Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R. M. L. Buller	Expert	LVD, NIAID
Others:	G. Palumbo	Staff Fellow	LVD, NIAID
	W. Chen	Visiting Fellow	LVD, NIAID
	G. Karupiah	Visiting Fellow	LVD, NIAID

COOPERATING UNITS (if any)

T. Fredrickson, U. of Connecticut; D. Pickup, Duke University; L. McIntyre, Vector Lab; T. Eling, NIEHS; Kerry Oliver, NIEHS; J. Burnett, U. of Maryland; R. Drillien, U. of Louis Pasteur, France; J. Kreider, Penn. State.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

5

PROFESSIONAL:

4

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

In this project, we focused our studies on the genetic basis both of orthopoxvirus virulence and of host resistance to virus infection. The acquired knowledge should contribute toward development of safe, effective recombinant vaccinia virus vaccines for animal and human use. A deletion mutant of cowpox virus (CPV) has been constructed which lacks a functional gene for a 38 kDa protein which directly or indirectly inhibits the generation of chemotactic molecules which are elicited during virus replication in the CAM. Current studies have shown that the 38-kDa protein specifically blocks the generation of 14,15-diHETE molecules in two separate cell lines infected with CPV. This protein will be evaluated for its therapeutic potential. Additional studies have shown that inhibitors of the lipoxxygenase pathway of arachidonic acid block the replication of orthopoxviruses as well as herpes simplex virus raising the possibility that this class of inhibitors may have broad anti-viral activity through an as yet to be determined mechanism.

Eighty percent of the ectromelia virus genome has been cloned. Three host-range genes have been sequenced and are being evaluated for their importance in defining virus cell, tissue and species tropism.

Studies on host resistance to disease have found that gamma interferon appears to be more important than alpha or beta interferon for recovery from infection with ectromelia virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00307-10 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Vaccinia Virus Gene Expression		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Moss	Laboratory Chief LVD, NIAID
Others:	B. -Y. Ahn	Visiting Associate LVD, NIAID
	B. Amegadzie	Visiting Fellow LVD, NIAID
	C. J. Baldick	Pre-IRTA LVD, NIAID
	P. Gershon	Visiting Associate LVD, NIAID
	J. Keck	NRC Fellow LVD, NIAID
	N. Harris	Visiting Fellow LVD, NIAID
	R. Rosales	Visiting Fellow LVD, NIAID
COOPERATING UNITS (if any) C. Winters, Walter Reed, Armed Focus Institute of Pathology; S. Broyles, Purdue University		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Genetic Engineering Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.2	6.7	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>Vaccinia virus has a genome of nearly 200,000 base pairs that encodes approximately 200 polypeptides. These genes are expressed within the cytoplasm in a coordinated fashion, so that some polypeptides are made before and others after DNA replication. Enzymes and factors needed for early transcription are packaged within the infectious particle, while those needed for intermediate and late transcription are present in the cytoplasm of infected cells. Vaccinia virus provides an excellent system for combining biochemical and genetic approaches to the study of transcription. The aim of this project is to determine the mechanisms regulating gene expression.</p> <p>This past year, progress has been made in defining the viral proteins involved in transcription and in understanding their roles. Emphasis has been on the virus-encoded RNA polymerase and transcription factors. Thus, three additional RNA polymerase subunit genes encoding polypeptides of 132,000, 35,000, and 30,000 Daltons were identified within the vaccinia virus genome and sequenced. All three genes are expressed both early and late in infection and the protein subunits are assembled into the RNA polymerase complex that is packaged in virions. Interestingly, the 30,000-Dalton subunit is homologous to a eukaryotic transcription elongation factor. One of the factors required for late transcription was shown to be encoded by an intermediate gene consistent with the cascade model of gene regulation. The promoter DNA contacts made by VETF, the vaccinia virus early transcription factor, were determined and evidence was obtained that this interaction leads to bending of the DNA.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00416-08 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recombinant Vaccines against Retroviruses Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Moss Laboratory Chief LVD, NIAID

Others: S. Chakrabarti Visiting Associate LVD, NIAID
P. Earl Microbiologist LVD, NIAID
V. Karacostas Pre-IRTA LVD, NIAID
E. Wolffe Special Volunteer LVD, NIAID

COOPERATING UNITS (if any)

LIR, NIAID, LI, NIAID; Metabolism Branch, NCI, LTCB, NCI; LCMS, NCI; Massachusetts General Hospital, Immuno AG; Molecular Vaccines, UpJohh Co.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS). At present there is no effective vaccine against this disease and therapeutic agents provide only limited help. The objects of this project are to characterize HIV antigens, determine the targets of humoral and cell-mediated immunity, and to use this information to develop candidate vaccines. We have constructed recombinant vaccinia viruses containing HIV genetic information. These viruses have been used as live experimental vaccines to immunize animals, to synthesize HIV proteins in tissue culture, to make targets for cytotoxic T cells, and study CD4-envelope protein interactions. A detailed kinetic and quantitative analysis of the early and late biosynthetic events undergone by the HIV-1 envelope glycoprotein expressed by a recombinant vaccinia virus was made. Studies with the drug brefeldin A suggested that assembly of gp160 into oligomers occurs in a post-golgi compartment. Chemical cross-linking, sucrose gradient sedimentation and polyacrylamide gel electrophoresis revealed that the HIV-2 envelope protein also assembles postranslationally into dimers and higher oligomers. The effects of C-terminal and internal deletions of the HIV-1 envelope protein on synthesis, transport, biological properties, and antigenicity were determined. The predominant conserved B cell epitopes were mapped to gp41 and the C-terminus of gp120 whereas cytotoxic T epitopes were distributed throughout the length of the glycoprotein. A new protease inhibitor was shown to block maturation of human and simian immunodeficiency viruses and spread of infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-00445-06 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Mechanisms of Viral DNA Replication		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI:	M. D. Challberg	Section Head LVD, NIAID
Others:	D. Fierer	Medical Fellow LVD, NIAID
	J. Gottlieb	IRTA Fellow LVD, NIAID
	G. Sherman	IRTA Fellow LVD, NIAID
	D. Klinedinst	IRTA Fellow LVD, NIAID
COOPERATING UNITS <i>(if any)</i> Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School; Department of Biochemistry, Medical College of Wisconsin		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Macromolecular Biology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>Herpes simplex virus is a useful model for studying the mechanisms involved in DNA replication in eukaryotic cells. Our current efforts are directed toward studying this process with purified proteins. Seven viral genes are both necessary and sufficient to carry out authentic origin-dependent DNA replication. Ongoing biochemical analyses in several laboratories support the idea that the products of these seven genes all participate directly in viral DNA synthesis. We are currently using both biochemical and molecular genetic approaches to understand the function of these polypeptides in detail.</p> <p>The HSV DNA polymerase purified from infected HeLa cells consists of a stable complex of two polypeptides: UL30, the catalytic subunit, and UL42, an accessory subunit. Several lines of evidence support the view UL42 increases the efficiency of the DNA polymerase by increasing its processivity. Using a novel synthetic model primer-template, we have characterized the interaction of the HSV DNA polymerase with its nucleic acid substrate. Our results suggest that UL42 increases the processivity of DNA polymerase by acting as a sliding clamp, reducing the probability that the polymerase will dissociate from the elongating DNA chain after each cycle of catalysis.</p> <p>The UL5, UL8, and UL52 polypeptides form a three protein complex that has both helicis and primase activities. Purification of the isolated subunits and subcomplex associations of subunits suggests that UL5 and UL52 act together to catalyze both helicase and primase activities. On the basis of sequence motifs, we presume that UL5 has the active site for helicase function, but UL5 is not active as a helicase in the absence of UL52. We propose that UL52 has the active site for primase function, and are trying to obtain direct support for this view. Although UL8 is required for neither helicase nor primase activities, it is required for the efficient utilization of primers by DNA polymerase in a model system for lagging strand synthesis. We suggest that the role of UL8 is to stabilize the association of nascent primers with the template, increasing the probability that primers are elongated by DNA polymerase. Work is underway to test this model.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00538-04 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interaction of Human Immunodeficiency Virus with the CD4 Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	E. Berger	Expert	LVD, NIAID
Others:	B. Moss	Laboratory Chief	LVD, NIAID
	C. Broder	NRC Associate	LVD, NIAID
	P. Robbins	Special Volunteer	LVD, NIAID

COOPERATING UNITS (if any)

I. Pastan, D. FitzGerald, NCI
L. Eiden, NIMH

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Genetic Engineering Section

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

2.1

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

CD4 is a surface molecule of human helper T-lymphocytes that serves as the receptor for human immunodeficiency virus (HIV), the causative agent of AIDS. We have focused on two areas related to CD4 and HIV:

1) Structure/function studies of the interaction of CD4 with the HIV-1 envelope glycoprotein (gp120/gp41 complex). The focus of this work is to identify structural changes in the envelope glycoprotein which occur upon CD4 binding, and which are involved in virion fusion with the cell membrane. Soluble CD4 was found to promote specific dissociation of gp120 from the envelope glycoprotein complex. Studies with synthetic peptide derivatives suggested that a particular region of CD4 (the CDR3 region) plays a critical role in inducing this structural change. Future efforts will focus on detailed analysis of the sites on CD4 and the envelope glycoprotein involved in fusion (using site-directed mutagenesis coupled with refined assays to measure membrane fusion). We have also made progress in developing approaches to detect conformational changes leading to exposure of the presumed fusogenic regions of the envelope glycoprotein.

2) Design of CD4-based therapeutics for the treatment individuals infected with HIV. We have continued to study a genetically engineered hybrid toxin (CD4-PE40) which is targeted to selectively kill HIV-infected cells. Previous work demonstrated potent anti-HIV activity against infected T-cells and monocyte/macrophages (primary cultures and continuous cell lines); the toxin was also shown to have highly synergistic effects with reverse transcriptase inhibitors. We have now found that CD4-PE40 is active against cells expressing diverse forms of HIV and SIV envelope glycoproteins, which differ in extent of processing, presence of a cytoplasmic tail, and binding affinity for CD4. These results suggest that the activity of CD4-PE40 may not be compromised *in vivo* by normal variations in envelope glycoprotein structure. We are now developing assays to test the activity of the hybrid toxin against primary isolates of HIV, including variants which have been shown to be highly refractory to neutralization by soluble CD4. We will also test newly developed CD4-PE40 constructs for possible enhanced anti-HIV activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00539-04 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Virus-Cell Interactions, Viral Pathogenesis, and Host Immunity		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	B. Moss	Laboratory Chief LVD, NIAID
Others:	S. Isaacs	Medical Staff Fellow LVD, NIAID
	R. Blasco	Visiting Associate LVD, NIAID
	Y. Zhang	Visiting Fellow LVD, NIAID
	H. Yuwen	Visiting Fellow LVD, NIAID
COOPERATING UNITS (if any) M. Frank and R. McKenzie, LCI, NIAID R. W. Doms, NCI		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Genetic Engineering Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	4.7	PROFESSIONAL: 4.2
		OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> A successful virus infection usually involves entry into the cell; uncoating, expression and replication of the genome; assembly and release of infectious virus particles; and defense against specific and non-specific host immune mechanisms. For this reason, some viral genes are required even for replication in tissue culture cells whereas others are only advantageous during animal infections. In a 4,500 base pair segment of the vaccinia virus genome, we found three genes that are homologous to the eukaryotic genes profilin (an actin binding protein), 3-β-hydroxysteroid dehydrogenase, and Cu-Zn superoxide dismutase. The role of the profilin homolog was examined by deleting the gene and characterizing the properties of the mutant. Surprisingly, the mutant was not defective in intracellular virus movement, formation of specialized microvilli or release of mature infectious virions. By contrast, deletion of another gene, encoding a protein of 37,000 Daltons that is a component of the outer envelope of vaccinia virus, resulted in a mutant that was defective in formation of extracellular virus and virus spread but produced normal amounts of infectious intracellular virus. Although the roles of non-essential genes can be studied by deletion mutagenesis, other methods are required for essential genes. We have used the regulatory elements of the Escherichia coli lac operon to construct a conditional lethal inducer-dependent virus. In this manner, we demonstrated the essentiality of an 11,000 Dalton core protein. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00540-04 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of Human Herpesviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Frenkel Supervisory Microbiologist LVD, NIAID

Others: B. Avidor Visiting Fellow LVD, NIAID
E. Roffman Visiting Associate LVD, NIAID
B. Lum IRTA Fellow LVD, NIAID
R. Danovich NRC Associate LVD, NIAID

COOPERATING UNITS (if any)

C. June, Tissue Bank, Navy

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

5

PROFESSIONAL:

4.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

Our studies concern the lymphotropic human herpesviruses 6 and 7 (HHV-6 and HHV-7). HHV-6 causes roseola infantum, a childhood disease characterized by high fever and skin rash. Human herpesvirus 7 (HHV-7) was isolated in our laboratory from CD4⁺ T cells of a health individual. Roseola infantum is generally a self limiting illness and virus can only be recovered during the acute phase of the disease. The virus then undergoes into a latent phase inasmuch as peripheral blood of healthy individual harbor viral DNA. Recent evidence has suggested that viral reactivation may occur in patients undergoing immunosuppression, such as in the course of bone marrow, liver, and kidney transplantation. Furthermore, the virus can cause serious disease, inasmuch as recent reports have provided evidence for viral involvement in central nervous system in roseola patients with encephalitic complications. It is thus of interest to study parameters which determine the interaction of the virus with the host cell, whether fully productive, persistent or latent. Our studies this past year concerned genome structure and the layout of viral genes, as a prelude to analyses of gene function and regulation. These studies included the cloning of 60 kb regions of HHV-7 DNA, sequencing close to 5 kb, mapping of HHV-6 DNA and analyses of heterogeneous viral DNA regions in a number of virus strains. A second avenue of research included studies related to virus-host cell interactions, with the goal of delineating parameters which affect and influence the course of infection in the human host. Studies in this category included analyses of the effect of T cell activation on virus replication, activation of HHV-6 and HHV-7 from latency, analyses of the effect of virus infection on T cell function, and analyses of parameters which determine the outcome of infection whether latent, recurrent, persistent or fully productive infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00541-04 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Folding Assembly and Transport of Viral Glycoproteins		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI:	J. W. Yewdell J. R. Bennink	Microbiologist Medical Officer (Research)
		LVD, NIAID LVD, NIAID
Others:	L. Eisenlohr	IRTA
		LVD, NIAID
COOPERATING UNITS <i>(if any)</i> T. Bachi, Institute for Immunology and Virology, Zurich R.P. Haugland, Molecular Probes, Eugene, OR M. Terasaki, LNNS, NINDS, NIH		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Viral Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.3	PROFESSIONAL: 1.16
		OTHER: .16
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided)</i> <p>The improvement of current antiviral vaccines and the development of novel vaccines depends on improving our understanding of viral attachment and fusion glycoproteins. Critical insight into the structure and function of viral glycoproteins is provided by studying their biosynthesis in virus-infected cells. We have studied the biosynthesis of influenza virus hemagglutinin. The HA is a critical antigen for protecting individuals against influenza, which remains a major cause of morbidity and mortality nationally and internationally. In addition, the fact that the HA is the best characterized viral glycoprotein allows it to serve as a model for elucidating general features of viral glycoprotein structure, function, and antigenicity.</p> <p>In the past year we have continued our studies on the biosynthesis of the influenza HA. Like many integral membrane glycoproteins, the mature HA is an oligomer, consisting of three identical monomeric subunits. We have used genetic and immunocytochemical techniques to determine where oligomerization occurs. Our findings are consistent with the idea that HA trimerization occurs in the intermediate compartment that exists between the endoplasmic reticulum and the Golgi complex. We have also continued our studies on the mechanism of action of brefeldin A (BFA). BFA is a fungal metabolite that blocks the exocytosis of secretory and integral membrane proteins. BFA was chemically coupled to a fluorescent compound (Bodipy) and the subcellular distribution of BFA-Bodipy determined by fluorescent microscopy. This revealed that BFA binds to membranes, and appears to have a particular affinity for the endoplasmic reticulum.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00542-04 LVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Processing of Viral Proteins for T Cell Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: J. R. Bennink LVD, NIAID
J. W. Yewdell LVD, NIAID

Others: J. Cox LVD, NIAID
L. Eisenlohr LVD, NIAID
F. Esquivel LVD, NIAID
C. Lapham LVD, NIAID

COOPERATING UNITS (if any)

N. Restifo, S. Rosenberg, COP, NCI
S. Marriott, J. Brady, LMV, NCI

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, MD 20892

TOTAL MAN-YEARS: 5.5	PROFESSIONAL: 4.8	OTHER: .66
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Class I molecules of the major histocompatibility complex class (MHC) consist of a highly polymorphic heavy chain complexed to B₂microglobulin. Class I molecules are expressed on virtually all cell types. Their sole function is to bind antigens and present them to T cells bearing CD8 molecules. These T cells are known as cytotoxic T lymphocytes (CTLs) due to their ability to lyse histocompatible cells in an antigen specific manner. CTLs play a critical role in eradicating intracellular pathogens and tumors. On the negative side, they are involved in organ rejection, and in many autoimmune dyscrasias.

There has been rapid progress in understanding the physical nature of the antigen-class I complex and in how antigens become associated with class I molecules in cells. Based in part on results from this laboratory, it is now apparent that antigens present in the cytosol are translocated into the exocytic compartment where they bind class I molecules which carry them to the cell surface for CTL recognition.

In the past year we have continued our studies on antigen processing, which can be defined as the structural modification and trafficking of protein antigens that enable the determinants recognized by CTLs (often buried in native proteins) to interact with MHC molecules in the proper subcellular compartment. We have focused on the following questions. In which exocytic compartment does association with class I molecules occur? Are there signals for targeting proteins into the cytosolic antigen processing pathway? Are there cellular proteins which function to facilitate the association of class I molecules with antigen? Can extracellular proteins be targeted to the cytosolic antigen processing pathway?

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00564-03 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Development of Parvovirus Vectors that Regulate Gene Expression		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> PI: J. A. Rose Section Head LVD, NIAID Others: S. Chatterjee Assistant Professor Dept. Microbiol., Georgetown U. K. Wong Senior Staff Fellow LVD, NIAID		
COOPERATING UNITS <i>(if any)</i> P. Johnson, Research Associate Professor, Georgetown University; V. Hirsch, Research Associate Professor, Georgetown University		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Structure Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.1	PROFESSIONAL: 2.1	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided)</i> <p> This study was designed to develop adeno-associated virus (AAV)-based vectors that could down-regulate expression of specific viral genes by mediating the production of appropriate antisense RNA molecules. Among targets chosen for study are certain early viral gene products which are absolutely necessary for productive viral infection. A series of AAV-based vectors has been created in which the endogenous AAV promoters and coding sequences were replaced with a polylinker and several strong constitutive and inducible heterologous promoters. An encapsidation system has been developed in order to produce high titres of infectious recombinant virus. The induction of resistance to both <u>H1V-1</u> and <u>HSV-1 infection</u> by such vectors is currently being studied, and present results indicate that a 95% reduction in the replication of either viruses can be achieved. Methods used include: <u>gene cloning</u>, <u>transfection</u> of cells, <u>radio-immunoprecipitation</u>, <u>immunofluorescence</u>, RNA and DNA <u>hybridization techniques</u>. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00619-01 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Intracellular Antibody-mediated Virus Neutralization		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i>		
PI:	J. W. Yewdell	Microbiologist LVD, NIAID
	J. R. Bennink	Medical Officer (Research) LVD, NIAID
Others:	R. Ffrench	Visiting Fellow LVD, NIAID
COOPERATING UNITS <i>(if any)</i>		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Viral Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS:	1.6	PROFESSIONAL: 1.33
		OTHER: .33
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided)</i> <p>Many antibodies specific for viral core proteins or non-structural proteins have the capacity to block viral infection if they are introduced into the cytosol of virus infected cells. Recent advances in understanding antibody folding indicate that antibodies have the ability to properly fold and bind antigen when expressed in the cytosol. Therefore, cells, or transgenic animals expressing a cytosolic form of an antibody capable of interfering with viral replication should be resistant to viral infection. Using electroporation to introduce antibodies into cells we have identified monoclonal antibodies that block infection with influenza viruses, and have begun to clone the corresponding genes from hybridoma cells with the goal of creating animals resistant to influenza virus infection.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00620-01 LVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular epidemiology of human herpesviruses 6 and 7		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	N. Frenkel	Supervisory Microbiologist LVD, NIAID
Others:	L. Wyatt	Senior Staff Fellow LVD, NIAID
COOPERATING UNITS (if any) K. Yamanishi, Osaka University, Japan; W. Rodriguez, Children's Hospital Washington DC; S. Strauss, NIAID, NIH; J. Burns, University of California, San Diego		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Molecular Genetics Section		
INSTITUTE AND LOCATION NIAID, NIH Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) HHV-6 and HHV-7 are newly recognized lymphotropic herpesviruses. HHV-6 is the causative agent of the childhood disease roseola infantum and recent evidence has suggested that it might be involved in infant hepatitis and in complex roseola cases with viral encephalitis. HHV-7 was isolated last year in our laboratory from CD4+ T cells of a healthy individual. Significant efforts this past year have been devoted to the development of epidemiological approaches and tests for these viruses. Examination of sera of healthy adults and children has shown that HHV-6 and HHV-7 are highly prevalent viruses which infect in early childhood. Seroconversion for HHV-7 occurs at a later age than seroconversion for HHV-6. Examination of virus isolates obtained from roseola patients as well as by reactivation of peripheral blood lymphocytes of healthy individuals, revealed that HHV-6 strains are highly conserved viruses, but they fall into two distinct groups of viruses which differ in their growth properties, restriction enzyme patterns, antigenic reactivity and disease association. Only one of these virus groups (prototyped by Z29 strain) appears to be associated with roseola infantum. It is as yet unknown whether viruses of the second virus prototyped by the U1102 strain infect in early childhood or are associated with any disease. In additional studies regarding disease association of HHV-6 and HHV-7 we have found no evidence for involvement of HHV-6/7 in Kawasaki Disease. Moreover, there was no compelling evidence for the involvement of HHV-6 or HHV-7 in chronic fatigue syndrome. Occasional elevated titers were noted but they may have reflected virus reactivation perhaps due to immune impairment in these patients. Studies designed to HHV-6/7 with additional diseases are in progress.		

LABORATORY OF INTRACELLULAR PARASITES
Rocky Mountain Laboratories
Hamilton, Montana
1991 Annual Report
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Annual Report
Laboratory of Intracellular Parasites
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1990 to September 30, 1991

Research Highlights

LICP scientists study infectious diseases caused by obligate intracellular bacterial pathogens. *Chlamydia trachomatis* and *Rickettsia rickettsii* infections are those studied primarily with research emphasis being directed towards defining and characterizing parasite surface components in relation to pathogenicity, virulence, and host immunity. The mechanisms used by intracellular parasites to infect their eucaryotic hosts and evade intracellular host defenses are being investigated. A major emphasis is placed on the identification and molecular characterization of parasite surface components that promote adhesion and invasion of host cells since they are logical targets for the development of experimental vaccines. Surface components that function as adhesions are being antigenically characterized at the molecular level in attempts to formulate rational approaches for the development of experimental vaccines to prevent or control infections caused by these intracellular pathogens; particularly sexually transmitted infections caused by *C. trachomatis*. Animal models are being used to define the role of host immunity in mediating chlamydial disease pathology. Chlamydial antigens which evoke deleterious immune responses are being characterized and the immune response(s) which promote immunopathology are being studied.

Chlamydia trachomatis: Harlan Caldwell and Hua Su of the LICP Immunobiology Section in collaboration with Michael Parnell, Chief of the Animal Care Section at RML, have completed phase I immunogenicity and protective efficacy studies using a synthetic peptide vaccine composed of key T_h and protective B-cell epitopes of the *Chlamydia trachomatis* major outer membrane protein (MOMP) in sub-human primates. Parenteral immunization of cynomolgus monkeys with the MOMP T:B cell chimeric peptide immunogen induced high titered chlamydial specific serum antibodies reactive with key protective B-cell determinants of the chimeric immunogen. Sera from vaccinated monkeys recognized the native MOMP and neutralized chlamydial infectivity *in vitro*. Vaccinated and control monkeys were challenged ocularly with *C. trachomatis* to determine the protective efficacy of the chimeric T:B cell peptide vaccine. Non-vaccinated monkeys developed a moderate/severe conjunctival hyperemia with distinct follicle formation that persisted for as long as 28 days post-challenge. In contrast, vaccinated monkeys developed a mild conjunctival hyperemia which resolved completely by day 14 post-challenge. Vaccinated monkeys shed less chlamydial organisms for a much shorter duration than the unvaccinated controls. Thus, parenteral vaccination with the chimeric T:B cell peptide did not prevent ocular infection but significantly reduced chlamydial shedding from the infected conjunctivae which was associated with less severe disease of a shorter duration. Elevated levels of chlamydial neutralizing antibody was found in tears of vaccinated monkeys as early as day 7 post-challenge. In contrast, tear antibody was not detected in unvaccinated controls until day 21 post-challenge. Thus, in vaccinated monkeys the early appearance of chlamydial neutralizing tear antibodies correlated directly with resolution of chlamydial infection. Tear

antibody in vaccinated monkeys was shown to be predominately IgG indicating that transudation of serum antibody had occurred following ocular infection. Thus, serum IgG antibody clearly had a protective effect, albeit partial, and was effective in resolving chlamydial ocular infection. It is likely that more solid resistance to chlamydial infection will provide stimulation of mucosal as well as systemic anti-MOMP neutralizing antibodies. Future work will focus on combined parenteral and oral vaccination protocols with chimeric MOMP T:B cell immunogens in the sub-human primate animal model in attempts to confer a more complete protective immunity against *C. trachomatis* infections of the oculogenital tract.

Richard Morrison and co-workers have continued investigations involving the pathogenesis of chlamydial disease. The chlamydial 60kD heat shock protein (HSP60) has been previously identified as a deleterious immune target and investigations this past year have focused on characterizing its antigenic properties. Ying Yuan and Morrison have prepared anti-chlamydial HSP60 monoclonal antibodies, identified the primary amino acid sequence to which they react, characterized their reactivities and specificities, and demonstrated their usefulness for the detection of chlamydia-infected cells and for the affinity purification of HSP60. The availability of specific antibodies and substantial amounts of purified chlamydial HSP60 provides a necessary component for our continuing studies involving this protein. Dan Rockey and Morrison have prepared MalE/HSP60 fusion polypeptides, that consist of portions of the chlamydial HSP60, expressed them in *E. coli*, and purified the recombinant fusion polypeptides. These purified fragments of the HSP60 will be used to localize antibody and T cell reactive regions of the molecule, which will provide important information regarding immunogenic sites on this molecule that stimulate pathogenetic immune responses. The antigenic stimulus for the severe inflammation associated with chlamydial disease has been proposed to come from cells persistently infected with chlamydiae. Persistent chlamydial infections can be established in vitro by culturing chlamydiae infected cells in the presence of penicillin G. Using this model of persistent infection, Morrison has shown that the synthesis of HSP60 is elevated compared to other chlamydial proteins such as the major outer membrane protein. Furthermore, HSP60 is detected in the culture supernatant, and is thought to be released from such infected cells. Morrison in collaboration with Gerald Byrne (Univ. Wisconsin) have shown that the immune mediator interferon- γ also induces persistent or cryptic infections in vitro, and that HSP60 synthesis is similarly elevated in these cells. If persistent infections occur in vivo, then these data suggest that such infected cells could function as depots for deleterious antigen. Future studies will focus on defining antibody and T cell regions of the HSP60, examining the human immune response to this protein, identifying subpopulations of T cells involved in the deleterious immune response, and characterizing the effect of interferon- γ on the induction of persistent chlamydial infections and defining how these infections might be involved in the pathogenesis of chlamydial disease.

***Rickettsia rickettsii*:** The newly created host-parasite biology unit under the direction of Ted Hackstadt has emphasized structure and function of rickettsial surface components. Significant progress has been made in defining gene structure of surface proteins that are the target of neutralizing antibodies. Bob Gilmore has completed sequencing of the rOmp B surface protein to reveal that this protein, with an apparent molecular mass of 135 kDa, is encoded by an open reading frame sufficient to encode a protein of 168 kDa. The rOmp B protein seems to be highly conserved among spotted fever group rickettsia and is antigenically

and structurally related to the more diverse typhus group rickettsiae. Hackstadt, in conjunction with Witold Cieplak (LVP) has determined the N-terminal amino acid sequence analysis of a 32 kDa surface protein to establish that it is encoded by the 3'-end of the rOmp B gene. The rOmp B gene product therefore appears to be proteolytically processed to yield two apparently stable outer surface exposed components. The putative precursor has not yet been detected on virulent rickettsiae but an avirulent mutant apparently fails to cleave rOmp B since it lacks the 32 kDa peptide fragment and displays a rOmp B protein of the size predicted for an uncleaved precursor. The 135 kDa rOmp B and the 32 kDa fragment appear to remain stably, but non-covalently, associated on the rickettsial surface since immunoprecipitation by monoclonal antibodies specific for the 135 kDa portion of the molecule precipitates both fragments. The association of these polypeptides on the rickettsial surface may be involved in maintenance of a proposed paracrystalline surface array. Paul Policastro has identified the transcriptional start site for the rOmp A and rOmp B messages and constructed plasmids with the rickettsial promoters controlling chloramphenicol acetyl transferase reporter genes to demonstrate that rickettsial promoters are highly active in *E. coli*. Primer extension analysis of the RNAs has confirmed that the promoters recognized by *E. coli* are the same as those recognized by actively growing rickettsiae. These studies will provide a rational basis for attempts to express rickettsial genes in recombinant hosts. Gilmore has also examined the gene structure of the rOmp A protein by restriction endonuclease mapping and Southern blot hybridization. A portion of the gene expressing up to 13 direct repeat units is conserved among all but one species of spotted fever group rickettsia. Diversity in gene structure is demonstrable, however, by restriction endonuclease cleavage patterns or by polymerase chain reaction product size even between closely related members of single species. Both the expressed proteins appear to be highly toxic to recombinant hosts. Efforts are continuing to express these proteins in alternate hosts for evaluation of their functional roles. Sita Awasthi has recently arrived and will be determining the role of phospholipase A in pathogenesis of spotted fever group rickettsiae through comparisons with avirulent mutants of *Rickettsia rickettsii* that fail to lyse infected cells.

ADMINISTRATIVE REPORT

Two new Sections have been added to LICP this year. The section of Immunobiology of Intracellular Parasites, formally the Chlamydial Diseases Section, is headed by Dr. Caldwell. LICP scientists working in this section are Dr. Morrison (GS-12 Microbiologist), Dr. Su (Visiting Associate), Dr. Manning (Staff Fellow), and Dr. Rockey (IRTA). Dr. Todd Cotter, a Ph.D. candidate in the Department of Microbiology and Immunology at the University of Michigan, has accepted a position in the LICP and will join the Section as an IRTA Fellow in February of 1992. The Host-Parasite Unit in the LICP is newly instituted and is headed by Dr. Hackstadt. LICP scientists working in the Unit are Dr. Policastro (Staff Fellow), Dr. Gilmore (Staff Fellow), Dr. Awasthi (Visiting Fellow), and Dr. Barry (IRTA Fellow). Drs. Awasthi and Barry are both new additions to the laboratory. Dr. Hackstadt has recruited two additional scientists to work in the Unit. Dr. Heinzen IRTA (Washington State University), and Dr. Brickman IRTA (University of Missouri) are scheduled to join the laboratory before the first of the new year. Summer students include returnees Yuan Ying, Mike Jasumback, Kathy Olson, Gwendolyn Lenk, Jennifer Rosquist and a first year student, Janelle McLean. The following people were invited to present seminars: Dr. Richard Stephens (University of California at San Francisco), Daniel D. Rockey (Oregon State University), Timothy J. Brickman (University of Missouri), Dr. Tom Jerrells (Univ. of Texas Medical Branch), Dr. Andrew Murdin (Connaught Laboratories, Willowdale, Ontario, Canada), Kim Weiss (University of Texas Medical Branch), Dr. Clifton Barry (Johns-Hopkins University), Dr. Svend Birkelund (University of California at San Francisco) Dr. Thomas Wynn (University of Wisconsin Medical School), Dr. Robert A. Heinzen (Washington State University), and Todd W. Cotter (Michigan State University).

HONORS AND AWARDS

NIH Honor Awards:

H. Caldwell - PHS Superior Service Award

Journal Editorial Boards:

H. Caldwell - Infection and Immunity (Editorial Board)
Proceedings of the National Academy of Sciences of (Invited Reviewer)
Journal of Immunology (Invited Reviewer)
Journal of Bacteriology (Invited Reviewer)
Microbiol Pathogenesis (Invited Reviewer)
Journal of General Microbiology (Invited Reviewer)

R. Morrison - Infection and Immunity (Invited Reviewer)

T. Hackstadt - Infection and Immunity (Invited Reviewer)
Journal of General Microbiology (Invited Reviewer)
Rev. Infect. Dis. (Invited Reviewer)

Professional Posts:

H. Caldwell - Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.

R. Morrison - Faculty affiliate, Division of Biological Sciences (Microbiology), University of Montana, Missoula, Montana.

Elected Post:

T. Hackstadt - Councilor-at-large, American Society for Rickettsia and Rickettsial Diseases.

Invited Lectures and Participation In Meetings and Symposia:

- H. Caldwell - Scientific Committee, Proceedings of the Seventh International Symposium on Human Chlamydial Infections, Harrison Hot Springs, British Columbia, Canada, June 24-29, 1990.
- R. Gilmore - American Society for Rickettsiology and Rickettsial Diseases, Galveston
American Society of Microbiology, Dallas, Texas.
- T. Hackstadt - American Society of Microbiology, Dallas, Texas
American Society for Rickettsiology and Rickettsial Diseases, Galveston, Texas.
- R. Morrison - Johns Hopkins University, Johns Hopkins Hospital, Baltimore, MD (1990)
National Institute of Allergy and Infectious Diseases, Science Days, STS Workshop, Bethesda, MD. (1990)
University of Wisconsin, Department of Medical Microbiology, Madison, WI. (1990)
American Society for Microbiology, annual meeting, Dallas, TX. (1991)
- P. Policastro - American Society for Rickettsiology and Rickettsial Diseases, Galveston, Texas. Participant.
- D. Rockey - American Society for Microbiology, Dallas, Texas.
- H. Su - American Society for Microbiology, Dallas, Texas.

Other Activities:

- H. Caldwell - NIAID Study Section, Bacteriology and Mycology, Ad hoc member.
NIAID Promotion and Tenure Committee
- R. Morrison - NIAID Study Section, Tropical Medicine and Parasitology, Ad hoc member.
NIAID Study Section, Bacteriology and Mycology, Ad hoc member.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00216-11 LICP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology of Chlamydial Surface Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Harlan D. Caldwell Chief LICP, NIAID

Others: Hua Su Visiting Associate LICP, NIAID
 Scott Stewart Microbiologist LICP, NIAID
 Jim Simmons Bio. Lab Tech. LICP, NIAID
 J.D. Sager Bio. Lab Tech. LICP, NIAID

COOPERATING UNITS (If any)

LAB/BRANCH

Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION

Immunobiology of Intracellular Parasites

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

5.5

PROFESSIONAL:

2

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

The overall goal of this project is the development of a vaccine to prevent or control blindness and infertility caused by *Chlamydia trachomatis*. The chlamydial major outer membrane protein (MOMP) is the principle target antigen for the development of a chlamydial vaccine. In past work, we have undertaken extensive studies aimed at defining the antigenic structure of the MOMP at the molecular level. Our objective was to identify structurally and functionally both helper-T (T_H) and protective B-cell epitopes of the MOMP that we believe to be key antigenic targets for the development of a synthetic or recombinant sub-unit chlamydial vaccine. From this work we identified an antigenically common T_H -cell MOMP epitope, that provides cognate help for the production of neutralizing antibodies to targeted serovar and conserved species-specific MOMP B-cell epitopes. Co-linearly synthesized chimeric T:B peptides corresponding to MOMP sequences containing the conserved T_H -cell epitope and serovar and species-specific B-cell epitopes induced functional chlamydial neutralizing antibody responses of the desired sero-specificity in strains of congenic mice differing at H-2. The chimeric peptides were also able to prime for neutralizing antibody responses following secondary challenge with sub-immunogenic quantities of intact chlamydiae. These immunogenic properties suggest that the synthetic T:B peptide immunogens may have considerable potential in chlamydial vaccine development.

To directly access the utility of the chimeric peptide immunogens as vaccine candidates we studied their immunogenicity and vaccine efficacy in a sub-human primate model of *C. trachomatis* ocular infection. Cynomolgus monkeys immunized parenterally with the synthetic peptide immunogens produced serum neutralizing antibodies but failed to produce detectable tear antibodies. Vaccinated monkeys were partially protected following ocular challenge with *C. trachomatis* in that they shed fewer chlamydiae and had significantly less disease. Partial protection was associated with a rapid appearance of neutralizing IgG antibodies in the tears of vaccinated monkeys which was likely localized to the eye by transudation. These findings are the first to demonstrate a beneficial effect of parenteral vaccination against *C. trachomatis* ocular infection using an antigenically defined immunogen. Future work will focus on combining parenteral and oral immunization protocols to stimulate both mucosal and systemic immunity in attempts to produce a more solid protective immunity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00519-04- LICP
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunopathogenesis of Chlamydial Infections		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	R.P. Morrison	Microbiologist LICP, NIAID
Others:	Dan Rockey K. Lyng	IRTA Chemist LICP, NIAID LICP, NIAID
COOPERATING UNITS (If any) Univ. Toronto, Ontario, Canada (R. Innam); London Sch. Trop. Med. and Hyg., London, United Kingdom (D. Mabey); Univ. Wisconsin Med. Sch., Madison, WI; New York Hosp.-Cornell Med. Ctr., New York, NY (S. Witkin); University of Montana, Division of Biological Science, Missoula, Montana (Y. Ying).		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">3</div>	PROFESSIONAL: <div style="text-align: center;">2</div>	OTHER: <div style="text-align: center;">1</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Infections caused by <i>C. trachomatis</i> primarily localize to ocular and genital tract mucosae where they commonly produce asymptomatic infections or acute self-limiting infections such as uncomplicated conjunctivitis, urethritis, or cervicitis. However, these infections occasionally progress to chronic infections that provoke severe inflammatory responses which may lead to blindness or infertility. <i>C. trachomatis</i> is the leading cause of sexually transmitted infection in the United States and Europe. In women, chlamydial infections of the lower genital tract can ascend to infect the fallopian tubes, produce chronic salpingitis, and cause infertility or ectopic pregnancy by tubal blockage. We previously identified the chlamydial 60kD heat shock protein (HSP60) as an antigen that elicits a local (mucosal) delayed hypersensitivity (DTH) response. We proposed that this immune response is pathogenetic and contributes to the development of the severe sequelae (blindness and infertility) that often follow chlamydial infection. The major focus of this project is to characterize the antigenic properties of the chlamydial HSP60, and to identify the immune responses elicited by this antigen that contribute to the pathogenesis of disease. This past year we have focused on preparing reagents that will allow us to evaluate the immune response to this antigen in detail. Anti-chlamydial HSP60 monoclonal antibodies were prepared. Eleven mAbs were obtained and their reactivities defined. Five mAbs were chlamydial specific and six cross-reacted with HSP60s from other gram-negative bacteria. One chlamydial specific mAb was useful for the affinity purification of HSP60, and all reacted with HSP60 by immunoblotting and were useful for the detection of chlamydial infected by indirect immunofluorescence. We have also prepared recombinant MalE/HSP60 fusion polypeptides that consist of truncated HSP60 polypeptides representing the entire primary amino acid sequence of the protein. These recombinant, truncated polypeptides will be useful for localizing the antibody and T cell domains of the HSP60 molecule. We are also utilizing in vitro models of chlamydial persistence to evaluate the possibility that cells persistently infected with chlamydiae act as antigenic depots for stimulation of chronic inflammatory responses. Interestingly, persistently infected cells contain elevated levels of chlamydial HSP60 compared to other chlamydial antigens, and preliminary evidence indicates that the chlamydial HSP60 is released from such infected cells. In the upcoming year we will define the T cell immune response to the chlamydial HSP60 and continue to evaluate the possibility that persistently infected cells function as depots of "deleterious" antigen. </p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 AI 00567-01 LICP

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Rickettsial Surface Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	Ted Hackstadt	Expert	LICP, NIAID
Others:	P.F. Policastro	Senior Staff Fellow	LICP, NIAID
	R.D. Gilmore	Staff Fellow	LICP, NIAID
	J.D. Sager	Bio. Lab. Tech.	LICP, NIAID
	R.E. Mann	Bio. Lab. Tech.	LICP, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Intracellular Parasites, Hamilton, Montana 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.5

PROFESSIONAL:

3

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

Rickettsia rickettsii is the causative agent of Rocky Mountain spotted fever. Strains of *R. rickettsii* and other species of rickettsiae differ considerably in virulence. We are applying modern molecular biological techniques to define and characterize these strains and attempting to identify virulence determinants on these obligate intracellular parasites. Current efforts are focused on a pair of high molecular weight surface proteins. These immunodominant surface protein antigens have estimated mol. masses of 190 and 135 kDa and are termed rOmp A and B, respectively. The DNA sequence of the gene encoding the rOmp B protein of *R. rickettsii* has been completed to identify a gene with the capacity to specify a protein of approximately 168 kDa. N-terminal amino acid sequencing of a 32 kDa outer membrane protein revealed that it is encoded by the 3' terminal end of the rOmp B gene suggesting that the rOmp B protein is proteolytically processed to yield the mature 135 kDa rOmp B protein and a 32 kDa fragment. Both polypeptides seem to be stable products and remain non-covalently associated on the outer surface of rickettsiae. Although we have not yet detected the rOmp B precursor on virulent *R. rickettsii*, analysis of an avirulent mutant of *R. rickettsii* revealed an absence of the 32 kDa fragment with a correspondingly larger rOmp B protein as would be predicted from an inability to cleave the expected precursor. The 190 kDa rOmp A protein of *R. rickettsii* (R strain) contains a large region of tandemly arranged repeats. Using a clone encoding this repeat region as a probe, homologous sequences were discovered within the genomes of all but one species of spotted fever group rickettsiae tested. Southern blot analysis on DNA fragments internal to a 3.8 kb PstI segment showed polymorphisms between species of Rickettsiae indicating structural differences among repeat regions. The coupling of PCR amplification with restriction endonuclease digestion has proven to be a rapid means to differentiate strains of *R. rickettsii*. Analysis of the promoter regions of the rOmp A and B genes by cloning the promoter in front of a chloramphenicol-acetyl transferase reporter gene revealed that these rickettsial promoters are active in *E. coli*. The activity of the promoters approximates the relative abundance of the respective proteins on rickettsia. This information will provide a rational basis for eventual attempts to express rickettsial genes in alternate hosts. Expression of rOmp A and B in recombinant hosts will be a powerful tool in definition of function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00612-01-LICP
PERIOD COVERED Beginning October 1, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression and Analysis of Recombinant Chlamydial Antigens		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	D. Scott Manning	Staff Fellow LICP, NIAID
Others:		
COOPERATING UNITS (If any)		
LAB/BRANCH Laboratory of Intracellular Parasites, Hamilton, Montana 59840		
SECTION Immunobiology of Intracellular Parasites		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)		
<p>This is a new project which focuses on the expression of the <i>Chlamydia trachomatis</i> major outer membrane protein (MOMP) in <i>Escherichia coli</i>. Evidence suggest that the MOMP serves as an adhesin and that protective immune responses are directed towards MOMP. The expression of MOMP in a system which is amenable to genetic manipulation would provide a useful tool for further studies of MOMP structure and function. Therefore, one of the goals of this project is to express MOMP in <i>E. coli</i> and to use site directed mutagenesis to study the contribution of specific amino acid sequences to structural, functional and antigenic properties. PCR amplification of MOMP sequences has been used to create several plasmids that express MOMP in <i>E. coli</i> cells. MOMP sequences have been fused to the signal peptide and amino terminal end of the <i>E. coli</i> OmpA protein.</p> <p>A second goal of this project is the use of <i>E. coli</i> for expression of MOMP B- and T-cell epitopes as fusions proteins with the B subunit of the <i>E. coli</i> heat-labile toxin (LT). Synthetic peptides which contain MOMP T-cell and B-cell epitopes in tandem have been shown to induce an anamnestic antibody response to the desired B-cell epitope (Su and Caldwell). The <i>E. coli</i> LT B subunit has a specific affinity for the GM1 ganglioside present on mucosal surfaces and has the unusual property of being a potent oral immunogen; thus, the expression of chlamydial epitopes as fusions proteins will facilitate targeting of the epitopes for stimulation of mucosal immunity. Construction and initial characterization of these fusions will be accomplished in the upcoming year.</p>		

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
1991 Annual Report
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Annual Report
LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1990, to September 30, 1991

RESEARCH HIGHLIGHTS

LMSF scientists focus their research on disease-causing microbes and their interactions with eukaryotic hosts. General goals are to decipher the infectious strategies and mechanisms employed by pathogenic microorganisms and those responses of the host that might be exploited for preventing or treating infections. Special attention is paid to genetic events that generate microbial variations, especially as they relate to avoidance and/or evasion of host defenses.

During FY 1991, LMSF staff members studied assorted aspects of *Neisseria gonorrhoeae* (Gc), *Borrelia burgdorferi* (Bb), group B streptococci (GBS), *Pseudomonas aeruginosa*, and human immunodeficiency virus (HIV). Other LMSF research utilizes more defined systems to explore basic questions about parasitic processes (including interspecies genetic transfer), replication of unusual forms of genomic DNA (such as linear plasmids), and molecular aspects of host defenses (including defensins, antibodies, and complement).

***Neisseria gonorrhoeae*:** Earlier studies in LMSF established that virulence of Gc is dependent on their having pili and that these organisms possess remarkable abilities to vary their pili *in vitro* and *in vivo*. Stuart Hill is exploring the contributions of DNA-transformation and intragenomic recombination to pilin subunit variation. He finds that both can produce alterations in the expressed pilin gene (*pilE*) and, hence, in pilin and pilus sequence. Although transformation of *pilE* is largely restricted to recipient cells with *pilE* sequence identical to that of incoming DNA, two-thirds of transformed Gc express an altered *pilE* because of temporally-linked recombinational modification with silent pilin gene (*pilS*) sequence.

Other Gc components, notably lipooligosaccharide (LOS) and colony opacity-associated outer membrane proteins (Opa), have implied but unproven pathogenetic functions. John Swanson has demonstrated that these outer membrane constituents cooperatively influence surface charge of Gc. Variant forms of these two molecules influence both surface charge and biological behaviors. LOS variant molecules with terminal galactose residues in their side chains incorporate sialic acid residues when Gc are supplied with cytidine-monophospho-N-acetyl-neuraminic acid (CMP-NANA), and such sialylation markedly changes both surface charge and susceptibility of outer membrane proteins to attack by exogenous proteases.

Bob Belland and Chen Tie have utilized *opa* genes previously cloned by Kolari Bhat to construct fusions between *bla* (β -lactamase) and *opa* which express Opa proteins at high levels on surfaces of *E. coli*. Such recombinant Opa+ *E. coli* exhibit physical and biological characteristics (cell surface charge, hydrophobicity, and interactions with human neutrophils or tissue culture cells) analogous to those of Gc that produce the same Opa (Steven Fisher, Belland, Chen Tie, and Swanson). The recombinant Opa appears to distribute and orient similarly in outer membranes of *E. coli* and Gc. The Opa+ *E. coli* can be used in several kinds of experiments that are impossible with Gc due to the potential of the latter to lyse and clump. Other *opa* recombinants in *E. coli* have been constructed to represent mosaics with variable portions from different *opa* genes; these are providing insight as to which domains of Opa contribute particular properties to the bacterial cell surface.

***Borrelia burgdorferi*:** Although *Borrelia burgdorferi* (Bb) is now known as the causative agent of Lyme disease and is susceptible to penicillin, major gaps persist in understanding the pathogenesis of acute Bb infections and their complications. A key question that has potential diagnostic and pathogenetic importance is whether Bb varies its surface components. Patti Rosa previously utilized polymerase chain reaction (PCR) with specially constructed oligonucleotide primers to identify two major groups of Bb that correlate quite well with their

geographic distribution; use of that technology allows detection of small numbers of Bb (less than 10) and has obvious diagnostic and research potentials. Rosa has recently found deletions and recombinational changes in Bb genes encoding two major outer membrane proteins (OspA, OspB); these generate novel Osp molecules that may represent functional analogues of the variable surface proteins of a related organism *Borrelia hermsii* (agent of relapsing fever). In collaboration with Tom Schwan (LVP), Rosa is exploring the relationships between these osp rearrangements and virulence of Bb. With Neil Margolis, she is examining the regulation of expression of key Bb proteins by Northern analysis.

An intriguing question of potential pathogenetic import is how Bb survives and multiplies in both a tick vector (at ambient temperatures) and in mammalian hosts (at 37°C). Kit Tilly has identified and molecularly cloned the Bb homologue of *dnaK*, and she has tentatively localized two genes that resemble *dnaJ* and *grpE* (of *E. coli*). These genes and their protein products are key "heat-shock" elements and likely have roles in Bb's ability to cope with changes in environmental temperatures. Tilly is also exploring methods for genetic transfer into Bb and the mode of replication of the novel linear plasmids. Steve Fischer, collaborating with Mark Klempner, has radiolabeled Bb with [⁷⁵Se]-methionine for studies on clearance and distribution of organisms following their inoculation into experimental animals.

Immunoglobulin biology: Group B streptococci (GBS) remain important causative agents of neonatal sepsis, but large gaps remain in understanding the factors that dictate virulence and pathogenicity of these organisms. Seth Pincus has identified colony opacity variants of GBS that differ in several ways including: chain formation, cell wall morphology, antibody binding, immunogenicity, complement activation, phagocytosis, and virulence in experimental animals. These variants switch from one to another by unknown mechanisms that are being explored.

Pincus previously designed and constructed immunotoxins that conjugate ricin A chain to monoclonal immunoglobulin molecules of desired specificities as potential agents for treating HIV infections; several directed against HIV envelope components were effective in eradicating HIV infection of tissue culture cells except when variant viruses arose. These variants have been molecularly analyzed by Pincus to better direct design of more effective immunotoxins. Recently Pincus purified anti-gp160 polyclonal antibodies from sera of HIV-positive individuals and found that these were even more effective in eradicating HIV infections in vitro when conjugated to ricin A chain. Pincus has also constructed appropriate immunotoxins against MuLV and, in collaboration with Leonard Evans (LPVD), is assessing their effectiveness against MuLV-induced neurodegenerative and neoplastic diseases.

Structure-function relationships of immunoglobulins are being explored by Carol Horgan through construction and study of recombinant genes that encode antibodies with desired antigenic specificities and altered hinge regions. These recombinant antibodies have proven useful for evaluating the function of antibodies within immune complexes; they have also been used to show that structure of the antigen-binding portion of an antibody influences its ability to fix complement. Horgan and Pincus have also cloned variable regions of anti-GBS antibody genes for constructing recombinant genes encoding antibodies with desired specificities against GBS.

Molecular aspects of microbial sex: Bacterial conjugation is now known to effect genetic transfers between species in differing biological kingdoms and is mainly responsible for spread of antibiotic resistances among diverse bacteria. Jack Heinemann has contributed significantly to that new understanding through his former demonstrations of mating between bacteria and yeast. He has more recently been dissecting the genetic transfer machinery by exploring mating between *Agrobacterium tumefaciens*, which causes crown gall formation in plants, and *E. coli* to identify those enterobacterial elements that are functional analogues to the well-characterized virulence-directing genes of the plant pathogens. Most recently, Heinemann has examined transfer of both heritable (DNA) and non-heritable (protein) components in *E. coli* by using a sensitive assay based on conversion of bacteriophage λ from a latent to an expressed state. His findings are that RecA protein can be transferred into recipient cells during conjugation and thereby can affect the heritable state of the recipient bacterial cell and its resident bacteriophage.

Synthetic defensin and other gene construction: Defensins are small polypeptides that are produced by mammals, particularly in professional phagocytic cells, and have potent antibacterial properties. Kolari Bhat has cloned and is sequencing a defensin-encoding gene from mouse DNA. Mutations in this gene, if the altered gene can be returned to intact animals, may provide important new insight into resistance to bacterial infections. Bhat has also devised a new method for assembling totally artificial genes of any desired sequences from a few, relatively long oligonucleotides synthesized *in vitro*.

ADMINISTRATIVE REPORT

Personnel changes in LMSF during FY91 include the departure of Osmar Barrera (GS-11, to NICHHD) who was replaced by Jeanne Wilson (from NCI). Kathy Messer (GS-7) also joined the technical staff. Robert Ankenbauer (from University of Washington) was recruited as an IRTA. Neil Margolis (from University of Pennsylvania) arrived as a Special Volunteer (FAES, Arthritis Foundation). Ralph Judd (University of Montana) was a Guest Worker for 3 months. Summer students included Greg Fischer (University of Pittsburgh School of Medicine), Bethany Tucker and Jason Shugart (Middlebury College), Jim Barry (Montana State University), and Robyn Hauser (Oregon State University). A national/international conference on Molecular Immunology of STD (non-AIDS) was held at RML, being hosted jointly by LMSF, LICP (RML), DOD, FDA, CDC, and the STD Branch, NIAID. Seminars were given by a number of outside guests: John Spitznagel (Emory University), Johanna Griffin (Boehringer Ingelheim Pharmaceuticals), Carol Hamilton (University of Chicago), Gregg Milligan (Washington University), Murty Madiraju (University of California, Berkeley), Costos Georgopoulos (University of Utah), Mark Robertson (USDA, Agricultural Research Service, Laramie), Patrick Moore (Center for AIDS Prevention Studies, San Francisco), Edward Berger (Laboratory of Viral Diseases, NIAID), Neil Margolis (University of Pennsylvania), and Karen Palter (Temple University, Philadelphia).

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Infection and Immunity
S. Pincus - medical advisory board for Arthritis Today

Manuscripts were reviewed by LMSF staff for the following journals: Canadian Journal of Microbiology, Cell, Infection and Immunity, Journal of Bacteriology, Journal of Biological Chemistry, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Journal of Rheumatology, Microbial Pathogenesis, Proceedings of the National Academy of Sciences USA, Science, and Sexually Transmitted Diseases.

Professional Posts:

S. Pincus - Adjunct Associate Professor of Internal Medicine, University of Utah,
Salt Lake City, Utah

P. Rosa - Member, Ad hoc Review Committee, NIH, DRG Bacteriology and Mycology-1 Study
Section, Bethesda, Maryland

J. Swanson - Advisor of Extramural NIAID-sponsored Sexually Transmitted Diseases
Cooperative Research Center, University of Texas Health Science Center
San Antonio, Texas
NIAID-MIDC Task Force on Microbiology and Infectious Diseases

Invited Lectures and Participation in Meetings and Symposia:

- J. Heinemann - Environmental Protection Agency, Corvallis, Oregon
EMBO Workshop on Bacterial Conjugation Systems, Schloß Ringberg, FRG,
Session Chairperson
- C. Horgan - Arthritis Foundation Fellows Conference, Snowbird, Utah
American College of Rheumatology, Seattle, Washington
American Association of Immunologists, Atlanta, Georgia
- S. Pincus - VII International AIDS Conference, Florence, Italy
University of Maryland, Baltimore, Maryland
Laboratory of Tumor Cell Biology, NCI, NIAID, Bethesda, Maryland
Workshop of the Intramural AIDS Targeted Antiviral Program, Bethesda,
Maryland
NIAID Symposium on Molecular Immunology of Sexually Transmitted Diseases,
Hamilton, Montana
- P. Rosa - American Society for Microbiology Annual Meeting, Dallas, Texas
Arthritis Foundation Fellows Conference, Snowbird, Utah
Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois
International Biotechnology Expo and Scientific Conference, San Francisco,
California
NIAID/NIAMS Scientific Workshop on Lyme Disease, NIH, Bethesda, Maryland
Banbury Conference--The Molecular Immunobiology of Lyme Disease, Cold Spring
Harbor, New York
- J. Swanson - University of Montana, Missoula, Montana
Max-Planck-Institut für Biologie, Tübingen, Germany
NIAID Symposium on Molecular Immunology of Sexually Transmitted Diseases,
Hamilton, Montana
- K. Tilly - Stress Proteins & the Heat Shock Response, Cold Springs Harbor, New York

Other Activities:

- J. Swanson - Reviewed research grants for National Science Foundation, Washington, DC
Consulted for Sexually Transmitted Diseases Branch, NIAID, Bethesda, Maryland

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00193-12 LMSF
PERIOD COVERED October 1, 1990, to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gonococcal Surface Components: Structure and Function		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	J. Swanson Others: S. A. Hill S. H. Fischer R. G. Ankenbauer O. Barrera S. G. Morrison J. M. Wilson	Chief Staff Fellow Research Associate IRTA Fellow Microbiologist Microbiologist Biologist LMSF, NIAID LMSF, NIAID LMSF, NIAID LMSF, NIAID LMSF, NIAID LMSF, NIAID
COOPERATING UNITS (if any) R. C. Judd, University of Montana, Missoula, MT J. M. Koomey, University of Michigan, Ann Arbor, MI		
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840		
SECTION 		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.85	2.9	2.95
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Studies on gonococcal surface components during the past year focused on mechanisms by which these bacterial pathogens alter the structure of their pili and on the effects that lipooligosaccharide (LOS) and opacity-associated (Opa) outer membrane protein constituents exert on gonococcal surface properties. Major recent findings include demonstration that pilin changes that follow transformation with DNA containing <i>pilE</i> (expressed pilin structural gene) are complex, often representing a mixture of transformation and intragenomic recombinations. Gonococcal surface properties are being explored in several ways, including use of Doppler electrophoretic light scattering technology to net cell charge. Both LOS and Opa constitution influence cell charge. The charges imparted by members of the repertoire of 10+ Opa proteins expressed by a single strain has been corroborated by examining <i>E. coli</i> that express the analogous but recombinant genes. LOS and Opa phenotypes also influence cell hydrophobicity. These physical characteristics of gonococci correlate relatively well to the phagocytosis of these organisms by human neutrophils <i>in vitro</i>. Additional studies in this laboratory include investigations by a Guest Worker (R. Judd) on a 44-kD protein that has penicillin binding properties in gonococci, and the beginning efforts of a newly-arrived staff member (R. Ankenbauer) to dissect the iron binding components and mechanisms of pseudomonas species. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00516-04 LMSF

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoglobulin Biology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. H. Pincus Expert LMSF, NIAID

Others: C. J. Horgan Senior Staff Fellow LMSF, NIAID
E. I. P. Kamanga-Sollo Visiting Fellow LMSF, NIAID
R. L. Cole Chemist LMSF, NIAID
K. Messer Bio Lab Tech LMSF, NIAID

COOPERATING UNITS (if any)

L. Evans, LPVD, NIAID
K. Brown, Special Volunteer, Arthritis Foundation

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

4.75

PROFESSIONAL:

3.0

OTHER:

1.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this work is to study the structure and function of the immunoglobulin molecule. The work is being performed so that this understanding will be applied to the development of antibodies for human therapy.

A. Therapeutic systems. 1. Antibodies to surface antigens of group B streptococci (GBS) have been demonstrated to have protective efficacy in a model of neonatal sepsis. We have identified colony opacity variants of GBS and studied their interactions with antibodies and other components of the immune system. 2. The efficacy of anti-HIV envelope antibodies coupled to ricin A-chain has been studied *in vitro*. Monoclonal and polyclonal antibodies directed against different epitopes have been tested. Biological variants of HIV that escape killing with these immunotoxins and CD4-PE40 have been identified. The phenotype of the cells carrying these HIV has been studied, and the molecular mechanisms of immunotoxin escape have been evaluated. We have also constructed anti-MuLV immunotoxins so that the efficacy of anti-retroviral immunotoxins may be tested *in vivo* in well-studied animal systems.

B. Genetically engineered antibodies. Vectors have been prepared carrying human constant region genes. These constructs contain both native and altered hinge region genes. These constructs have been ligated to variable region genes from antibodies to the synthetic polypeptide (Tyr.Glu)-Ala--Lys. Antibodies were expressed and purified. Well-defined immune complexes were constructed using antigens having different epitope densities. The ability of the immune complexes to interact with the complement system is being studied. We are currently cloning and expressing anti-GBS antibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00549-03 LMSF

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Infection with the Lyme Disease Spirochete, *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. A. Rosa Senior Staff Fellow LMSF, NIAID

Others: K. L. Tilly Senior Staff Fellow LMSF, NIAID
S. H. Fischer Research Associate LMSF, NIAID
D. M. Hogan Microbiologist LMSF, NIAID

COOPERATING UNITS (if any)

N. Margolis, Special Volunteer, Arthritis Foundation
T. G. Schwan, NIAID, RML, Laboratory of Vectors and Pathogens
M. Klempner, Tufts University College of Medicine

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.3

PROFESSIONAL:

2.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Infection of humans by *Borrelia burgdorferi* can result in a multisystemic, chronic disease with diverse manifestations. We are investigating *B. burgdorferi* at several levels in order to understand some aspects of the disease pathogenesis. These studies include genetic manipulations of the spirochete, characterization of key structural and regulatory elements, and dissemination and clearance of spirochetes in mammalian hosts.

1. Molecular Characterization: Several features of *B. burgdorferi* are being addressed. Recombination between the genes encoding the major outer surface proteins (OspA, OspB) of *B. burgdorferi* can result in both the deletion and generation of *osp* gene sequences. Such recombinants have been detected and characterized in a number of strains. The molecular basis of additional Osp phenotypes is being investigated. Isolating and studying genes whose expression is increased after a temperature upshift (heat shock genes) will provide information about their roles in cell growth, pathogenesis, plasmid replication and adaptation to stress. Understanding the mechanism of linear plasmid replication may yield a new target for antibiotics, in addition to shedding light on ways in which organisms manipulate their genetic material.

2. Gene Transfer: In order to allow genetic analysis of *B. burgdorferi*, a method for gene transfer into *Borrelia* is being developed. A derivative of the *Borrelia* 16 kb linear plasmid will be used as a vector for electroporation, allowing for maintenance of transferred DNA in a native setting.

3. Clearance Studies: Mechanisms that allow *B. burgdorferi* to evade host defenses to establish chronic infections in a variety of host tissues remain obscure. During natural infections of mammals, *B. burgdorferi* must first disseminate from the cutaneous site to other tissues and organs. A mouse clearance model has been developed that makes it possible to quantitatively evaluate the distribution and clearance of intravenously injected *B. burgdorferi*. It may be possible to correlate patterns of clearance with different structural features of cloned organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00568-02 LMSF

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis and Extent of Microbial Sex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. A. Heinemann

IRTA Fellow

LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bacterial conjugation can promote genetic exchange between biological kingdoms and is largely responsible for the evolution of antibiotic resistances among eubacteria. Genetic exchange between bacteria, and between bacteria and their hosts, promotes pathogenesis, such as *Agrobacterium tumefaciens* induced tumorigenesis in plants. Even when transferred genes are not inherited, the transfer of DNA and protein between cells may have a heritable effect. Transferred molecules of DNA can serve as templates for the repair of damaged DNA in the recipient, and transferred proteins may alter an existing epigenetic state. Such exchanges are important, therefore, for two reasons: assessing the extent and impact of genetic exchange in pathogenesis and determining the potential for altering a cellular program through the exchange of non-genetic material. I have been studying the molecular factors which accompany transfer of genetic material during conjugation. These factors are particularly intriguing because they are required primarily or specifically in the recipient organism, may be encoded by the donor cell chromosome rather than the virulence factors, and are required in a species-specific manner. I have found that the conjugal transfer of one co-factor, the RecA protein of *Escherichia coli*, can trigger the release of latent phage. The transfer of a chromosomal protein can therefore affect a heritable state of both cell and phage and may have implications for the development of a disease susceptibility in humans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 AI 00608-01 LMSF									
PERIOD COVERED October 1, 1990, to September 30, 1991											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Expression and Phase Variation of Gonococcal opa Genes											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: R. J. Belland </td> <td style="width: 33%; vertical-align: top;"> Visiting Associate </td> <td style="width: 33%; vertical-align: top;"> LMSF, NIAID </td> </tr> <tr> <td colspan="3" style="padding: 5px 0 0 0;">Others:</td> </tr> <tr> <td style="vertical-align: top;"> T. Chen S. H. Fischer J. Swanson S. G. Morrison </td> <td style="vertical-align: top;"> Visiting Fellow Research Associate Chief Microbiologist </td> <td style="vertical-align: top;"> LMSF, NIAID LMSF, NIAID LMSF, NIAID LMSF, NIAID </td> </tr> </table>			PI: R. J. Belland	Visiting Associate	LMSF, NIAID	Others:			T. Chen S. H. Fischer J. Swanson S. G. Morrison	Visiting Fellow Research Associate Chief Microbiologist	LMSF, NIAID LMSF, NIAID LMSF, NIAID LMSF, NIAID
PI: R. J. Belland	Visiting Associate	LMSF, NIAID									
Others:											
T. Chen S. H. Fischer J. Swanson S. G. Morrison	Visiting Fellow Research Associate Chief Microbiologist	LMSF, NIAID LMSF, NIAID LMSF, NIAID LMSF, NIAID									
COOPERATING UNITS (if any) Wai-Mun Huang, University of Utah, Salt Lake City, Utah											
LAB/BRANCH Laboratory of Microbial Structure and Function, Hamilton, MT 59840											
SECTION											
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892											
TOTAL MAN-YEARS: <div style="text-align: center; font-weight: bold;">2.6</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">2.3</div>	OTHER: <div style="text-align: center; font-weight: bold;">0.3</div>									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"><input type="checkbox"/> (a) Human subjects</td> <td style="width: 33%;"><input type="checkbox"/> (b) Human tissues</td> <td style="width: 33%;"><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td></td> <td></td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The major surface components of the human pathogen <i>Neisseria gonorrhoeae</i> change dramatically during the course of gonorrheal disease in male volunteers. Changes involve the expression states of particular genes in conjunction with the genetic content of the genes being expressed. The Opa family of outer-membrane proteins change expression states as a result of a unique translational switching mechanism involving the insertion or deletion of variable numbers of pentameric coding repeat elements (CR) within that segment of the gene coding for the signal-peptide of the Opa pre-protein. The direct, tandem repeat elements have the capacity to form an intramolecular triplex (H-DNA) in response to negative supercoiling torsion. The <i>opa</i> CR region is the first naturally occurring prokaryotic DNA sequence shown to adopt this non B-DNA conformation and is also unique in that the sequence occurs within the coding sequence of the gene. The phase variation frequencies of repeated genetic elements which form H-DNA are significantly lower than elements with the same repetitive nature which are unable to form H-DNA. Transcription of both types of repeat elements results in increased phase variation frequencies, but this effect is less pronounced in H-DNA forming repetitive sequences. Transcription of <i>opa</i> genes is constitutive for the 5' end of the gene but shows a strong termination when the genes are in the "off" configuration. The termination may be linked to the formation of the H-DNA structure and if so, represents a novel termination mechanism which could allow the cell to conserve nucleotide triphosphates. The enzymes responsible for generation of negative supercoiling (<i>gyrA/B</i>) were cloned and are being purified to determine their effect on the transcription of <i>opa</i> and the relationship between transcription and H-DNA formation. A number of Opa proteins have been constitutively expressed as β-lactamase-Opa fusions in <i>E. coli</i> and have been shown to segregate to the outer-membrane in a conformation resembling that seen in <i>N. gonorrhoeae</i>. Recombinant Opa⁺ bacteria mimic the interactions seen between human neutrophils and gonococcal strains expressing the analogous Opa. Recombinant strains also show an ability to adhere to certain eukaryotic cell lines in <i>in vitro</i> binding assays. </p>											

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1991 Annual Report
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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1989 to September 30, 1990

ADMINISTRATIVE REPORT

The following staff changes occurred at LPVD this past year:

Arrivals:

- Dr. Suzette Priola, IRTA Fellow, from NIH Training Grant working with Dr. Bruce Chesebro.
- Dr. Kim Hasenkrug, IRTA Fellow, from Albert Einstein College of Medicine, Bronx, New York working with Dr. Bruce Chesebro.
- Dr. Mutsumi Satoh, Visiting Fellow, from Sapporo Medical College working with Dr. John Coe.

Departures:

- Dr. Michael N. Robertson, Medical Staff Fellow, going to work in the laboratory of Professor Luc Montagnier, Institute of Pasteur, Paris, France.
- Dr. Markus Czub, Visiting Fellow, returning to work at the Institute of Virology and Immunology, University of Würzburg, Germany.
- Dr. Stephanie Czub, Visiting Fellow, returning to work at the Institute of Pathology, University of Würzburg, Germany.
- Summer students were Bradley Berry from Carroll College, Helena, MT; John R. Courchesne from University of Montana, Missoula, MT; Michelle Loftis from the University of Nebraska, Omaha, NE; James Nolz from University of Nevada, Reno, NV; Cotton Seed from Corvallis, MT; and Jessica Walker from Swarthmore College, Swarthmore, Pennsylvania.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00072-20 LPVD															
PERIOD COVERED October 1, 1990 to September 30, 1991																	
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice																	
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: D. L. Lodmell</td> <td style="width: 33%;">Scientist Director</td> <td style="width: 33%;">LPVD, NIAID</td> </tr> <tr> <td>Other: Linda L. Perry</td> <td>Expert</td> <td>LPVD, NIAID</td> </tr> <tr> <td>Joseph J. Esposito</td> <td></td> <td>CDC</td> </tr> <tr> <td>William J. Bellini</td> <td></td> <td>CDC</td> </tr> <tr> <td>John W. Sumner</td> <td></td> <td>CDC</td> </tr> </table>			PI: D. L. Lodmell	Scientist Director	LPVD, NIAID	Other: Linda L. Perry	Expert	LPVD, NIAID	Joseph J. Esposito		CDC	William J. Bellini		CDC	John W. Sumner		CDC
PI: D. L. Lodmell	Scientist Director	LPVD, NIAID															
Other: Linda L. Perry	Expert	LPVD, NIAID															
Joseph J. Esposito		CDC															
William J. Bellini		CDC															
John W. Sumner		CDC															
COOPERATING UNITS <i>(if any)</i> None																	
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840																	
SECTION																	
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892																	
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0															
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<input type="checkbox"/> (a1) Minors																	
<input type="checkbox"/> (a2) Interviews																	
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided..)</i> <p>The principal objectives of this research project are two-fold: 1) to determine host and viral factors that influence the genetically controlled resistance of inbred mouse strains to street rabies virus (SRV) and 2) to determine the mechanism(s) by which recombinant vaccines expressing different structural proteins of the rabies virus protect highly susceptible strains of mice against lethal infection.</p> <p>In a continuation of our cytotoxic T lymphocyte (CTL) studies, we have utilized monoclonal antibodies specific for CD4⁺ or CD8⁺ subsets of T cells to deplete the respective cell population in mice of the SJL/J and BALB/cByJ strains that are naturally resistant to SRV. Elimination of CD4⁺ T-helper cells abrogated the production of immunoglobulin G (IgG) neutralizing antibodies that occurs during rabies virus infection and reversed the resistant status of SJL/J and BALB/cByJ mice. In contrast, <i>in vivo</i> depletion of CD8⁺ cytotoxic T cells had no measurable effect on host resistance to SRV.</p> <p>Studies with recombinant vaccines expressing rabies virus structural proteins have shown that SRV susceptible mice vaccinated with raccoon poxvirus (RCN) recombinant viruses expressing the nucleoprotein (N) of the rabies virus are resistant to lethal challenge with SRV. Maximum survival was achieved following vaccination by tail scratch and footpad (FP) challenge. Mice immunized intraperitoneally were not protected. RCN-N-vaccinated mice inoculated in the FP with SRV were resistant to infection for at least 54 weeks postvaccination. Protection was also elicited by RCN recombinants expressing the rabies virus glycoprotein (RCN-G). Vaccination with RCN-G evoked rabies virus neutralizing antibody. Rabies virus neutralizing antibody was not detected in RCN-N-vaccinated mice prior to or following SRV infection. Sera from RCN-N-vaccinated mice which survived SRV infection did not contain antibody to SRV structural protein G, M or NS. The mechanism(s) of N-induced resistance correlates with the failure of peripherally inoculated SRV to enter the central nervous system (CNS). Support for this correlation with resistance was documented by the observations that SRV-inoculated RCN-N-vaccinated mice did not develop clinical signs of CNS rabies virus infection, infectious SRV was not detected in the spinal cord or brain following FP challenge, and 100% of RCN-N-vaccinated mice died following direct infection of the CNS with SRV via the intranasal or intracranial route. To date we have determined that the resistance of N-vaccinated mice does not appear to be due to interferon, NK cells, cytotoxic antibody, CTLs, or antibody-dependent-cell-mediated cytotoxicity. The search for the mechanism(s) of resistance continues. These results suggest that factors other than anti-G neutralizing antibody are important in resistance to rabies virus and that the N protein, because of its remarkable sequence conservation among N proteins of different rabies viruses, should be considered for incorporation with the G protein in recombinant vaccines. We are continuing to investigate the importance of CTLs in resolving rabies infections within the CNS, the mechanism(s) of immunity induced by recombinant vaccines expressing the rabies virus N, and the possibility that anti-rabies virus antibody localized within neurons and microglia affects the outcome of infection.</p>																	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00074-19 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetically Controlled Mechanisms of Pathogenesis and Recovery in Friend Retrovirus-Induced

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Chesebro Chief LPVD, NIAID

Other: M. Miyazawa Visiting Associate LPVD, NIAID
M. Robertson Medical Staff Fellow LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

2.7

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

This project is focussed on the mechanisms of pathogenesis and immunity involved in Friend murine retrovirus-induced leukemia. In mice inoculated neonatally with Friend murine leukemia helper retrovirus two distinct pathogenic mechanisms have been identified: early hemolytic anemia with increased erythropoiesis and erythroleukemia with blocked erythropoiesis. Recent results using viral variants have shown that hemolytic anemia is altered in viral clones with a single amino acid difference in the envelope protein. Similarly lack of a second direct repeat enhancer sequence in the viral LTR decreased the anemia. These results should lead to a better understanding of the mechanisms by which retroviruses lyse infected cells in vivo.

Studies on the role of the host immune system in protection of mice against Friend virus leukemia have focussed recently on the roles of different viral protein as vaccines. Purified viral envelope glycoprotein was found to be the best virion protein immunogen. In association with a strong adjuvant this glycoprotein was capable of protecting both H-2^{a/b} and H-2^{a/a} mice. Protection of H-2^{a/a} was surprising because these mice were not protected by vaccinia virus expressing Friend virus envelope protein. The results indicated that purified denatured envelope protein plus complete Freund's adjuvant induced both a T helper cell response and accelerated switching of anti viral antibodies from the IgM to IgG isotype.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00085-14 LPVD									
PERIOD COVERED October 1, 1990 to September 30, 1991											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogenesis of Aleutian Disease Virus Infection											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: M. E. Bloom</td> <td style="width: 33%;">Medical Officer</td> <td style="width: 33%;">LPVD, NIAID</td> </tr> <tr> <td>Other: H. Kanno</td> <td>Visiting Fellow</td> <td>LPVD, NIAID</td> </tr> </table>			PI: M. E. Bloom	Medical Officer	LPVD, NIAID	Other: H. Kanno	Visiting Fellow	LPVD, NIAID			
PI: M. E. Bloom	Medical Officer	LPVD, NIAID									
Other: H. Kanno	Visiting Fellow	LPVD, NIAID									
COOPERATING UNITS (if any) Department of Pathology, Tohoku University School of Medicine, Sendai, Japan											
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840											
SECTION											
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892											
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1.5	OTHER: 0.5									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td colspan="2">This is a non-clinical IIDEA project.</td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews	This is a non-clinical IIDEA project.	
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews	This is a non-clinical IIDEA project.										
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The scope of this project is to elucidate pathogenic mechanisms involved in infections of mink with Aleutian mink disease parvovirus (ADV).</p> <p>In the past year, we developed a method for in situ hybridization using nonradioactive probes. Nonradioactive probes provide a clearer picture of cellular morphology than radioactive probes, although they are noticeably less sensitive. Using both probe types, we have attempted to identify more closely the target cells for ADV replication in adult mink. Our previous work indicates the target cells are found in lymph nodes and have the distribution of follicular dendritic cells and macrophages. Using probes specific for virion DNA sequestration, we localized virion sequences in both macrophages and follicular dendritic cells. However, using conditions specific for ADV mRNA, ADV replication could be conclusively localized only to macrophages.</p> <p>Attempts to isolate macrophages and follicular dendritic cells from infected mink lymph nodes yielded low numbers of cells with poor viability. Furthermore, extremely low numbers of ADV infected cells could be found in these populations as assessed by in situ hybridization and the type cell could not be defined.</p> <p>Cells from the peritoneal cavity of ADV infected mink were also studied. Cells containing ADV nucleic acid were readily identified. The number of cells containing virion DNA was about 10 times that of cells with ADV mRNA. Some of these cells were phagocytic, supporting the notion that macrophages are target cells for ADV. Finally, adherent resident peritoneal cells from normal mink were infected in vitro with ADV and a small number of these cells replicated ADV as assessed by the production of ADV antigens.</p> <p>In other work, we began to study the transcription program of ADV in vivo. Northern blot analysis of mRNA from the lungs of infected mink kits suggested that ADV transcription in this tissue is similar to that observed in permissive in vitro infections. mRNA from the lymph nodes and peritoneal exudate cells of infected adult mink also had detectable ADV mRNA, but the amount was low. Nevertheless, all ADV mRNA species appeared to be present.</p>											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00086-14 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Diseases Induced by Non-Oncogenic Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. L. Portis Medical Officer LPVD, NIAID

Other: Markus Czub	Visiting Fellow	LPVD, NIAID
William Lynch	IRTA	LPVD, NIAID
Stephanie Czub	Visiting Fellow	LPVD, NIAID
Louis Qualtiere	Special Volunteer	LPVD, NIAID

COOPERATING UNITS (if any)

Dr. Lennert Mucke and Dr. Michael Oldstone, Scripps Clinic and Research Foundation, La Jolla CA.

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

5.2

PROFESSIONAL:

4.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

The retroviruses we study were isolated from wild mice and cause a non-inflammatory neurodegenerative disease in mice similar to that caused by the unconventional agents such as scrapie, but this disease does not involve the generation of "prion" protein. We are using this system as an animal model for studying the pathogenesis of retrovirus-induced neurodegeneration. This laboratory has divided its efforts into three areas: Identification of the viral sequences which mediate tempo and character of the neurovirulence; the host factors which are involved in susceptibility to disease; and the mechanisms involved in the induction of neuronal cytopathology. In the past year we have made considerable headway in all three areas. The disease as it appears in feral mice has a long incubation period of up to 1 year. Using molecular cloning techniques we have identified a 41 base sequence in the 5' leader of the viral genome which appears to be a primary determinant of incubation period. Initial studies indicate that this sequence functions by influencing the efficiency of virus spread *in vivo*. This sequences resides within an open reading frame for a glycosylated form of the *gag* polyprotein. Host factors are also involved in the determination of incubation period. We previously found that the CNS is susceptible to infection until the 10th day of age. We have recently found that the length of the incubation period is dependent on a dynamic relationship between this progressive age-dependent restriction of virus replication in the CNS and the rate of virus replication within peripheral organs. This finding has provided considerable insight into the host/virus interactions which determine the kinetics of this "slow virus" disease. We are continuing our efforts to uncover the pathogenetic mechanisms responsible for the neuronal cytopathology induced by the wild mouse retrovirus. Using microinjection techniques we were able to increase the number and expand the types of cells in the CNS which were infected. Yet the course of the disease was not changed from that seen in neonatally inoculated mice. Thus, it appears that the neurodegeneration induced by this virus requires that a particular stage of postnatal CNS development be attained. This observation, in addition to our previous finding that infected neurons exhibit no evidence of cytopathology and neurons exhibiting cytopathology appear not to be infected, suggest that the neurodegeneration is an indirect consequence of virus infection. Future research goals involve the search for a possible neurotoxin using known inhibitors of NMDA, kainate and quinolinic acid receptors. In addition, we plan to examine the possible role of microglial cell activation in this disease. We will continue our studies of the viral sequences which mediate neurovirulence. Identification of these sequences and their respective function should provide further insight into the pathogenesis of this neurodegenerative disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00260-10 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Chesebro Chief LPVD, NIAID

Other: J. Portis Medical Officer LPVD, NIAID

L. Evans Chemist LPVD, NIAID

COOPERATING UNITS (if any)

Dr. M. Sitbon, Hopital Cochin, Paris, France

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.0

PROFESSIONAL:

0.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical AIDS-related project.

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

This project has been terminated. It has been combined with project 074.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00262-10 LPVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Role of Pentraxins in Acute and Chronic Pathology		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)</i> <div style="display: flex; justify-content: space-between;"> PI: J. E. Coe Medical Officer LPVD, NIAID </div> Other:		
COOPERATING UNITS <i>(if any)</i> Drs. B. Canquihem & P. Pevet, Strasbourg, France; Dr. B. Dowton, Wash. Univ. Med. School, St. Louis, MO; Dr. K. Ishak, AFIP, Washington, D.C.; Dr. U. Nilsson, Uppsala, Sweden; Dr. Mortensen, OSU, Columbus, OH; Dr. D. Johnson, U. Kansas Med. Center, Kansas City; Dr. Gary Nelsestuen, U. Minn., St. Paul; Dr. J. Ward, NCI, Ft. Dietrick, MD; Dr. T. Duclos, U. New Mex., Albuquerque, NM; Dr. H. Gewurtz, Rush Med. Center, Chicago, IL		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION NIAID, NIH, Bethesda, MD 20892		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided..)</i> <p> Steroid hormones have some unusual effects in hamsters. We have been studying the endocrine control of a protein synthesized in liver of the Syrian hamster. This protein is homologous to human C-Reactive protein (CRP) and amyloid P component (AP) sharing a 50-70% (respectively) identical amino acid sequence and having a similar molecular configuration; that is, an oligomer of 5 identical monomer proteins noncovalently assembled as a cyclic pentamer. Furthermore, FP shares similar functional properties with CRP + SP such as Ca⁺⁺ dependant Phosphoryl-choline and galactan binding, complement fixation, and acute phase responsiveness. We have previously shown that FP is a constituent of hamster amyloid, and high serum levels of FP appear to have a primary role in the deposition of amyloid in the Syrian hamster. So far, only in hamster is pentraxin synthesis controlled by sex hormones so that females have 200-300 fold more FP in serum than males. This unusual endocrine control suggests that FP in the hamster may have some unusual function which could provide information about the general function for all pentraxins. We have examined other members of the hamster family to determine if endocrine control of pentraxin synthesis is a general feature in these rodents. FP synthesis in Armenian hamster is under a very different control mechanism, because estrogen administration actually decreases FP synthesis in direct contrast to effect of estrogens in Syrian (male) hamsters. Of particular interest is the acute hepatotoxicity induced in Armenian hamsters by exogenous estrogen which is detectable histologically by hepatocellular degeneration. Chronic administration of diethylstilbestrol (DES) is associated with the appearance of hepatocellular carcinomas. Recently we have observed the formation of neoplastic lesions in liver after chronic treatment with Zeranol, an estrogen produced by fungi and frequently found as a contaminant of food. Although Zeranol is known as a relatively weak estrogen, the effect of Zeranol on liver of Armenian hamster is much greater than expected. The indication of hepatic tumors by estrogen alone is unusual, and the Zeranol-hamster model is a particularly suitable experimental animal for studies of hormone-initiated carcinogenesis. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00263-10 LPVD									
PERIOD COVERED October 1, 1990 to September 30, 1991											
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Structure and Function of the ADV Genome											
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: M. E. Bloom</td> <td style="width: 40%;">Medical Officer</td> <td style="width: 20%;">LPVD, NIAID</td> </tr> <tr> <td>Other: Dahn Clemens</td> <td>IRTA</td> <td>LPVD, NIAID</td> </tr> </table>			PI: M. E. Bloom	Medical Officer	LPVD, NIAID	Other: Dahn Clemens	IRTA	LPVD, NIAID			
PI: M. E. Bloom	Medical Officer	LPVD, NIAID									
Other: Dahn Clemens	IRTA	LPVD, NIAID									
COOPERATING UNITS <i>(if any)</i> Department of Veterinary Virology, Royal Danish Veterinary University, Copenhagen, Denmark											
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840											
SECTION											
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892											
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1.5	OTHER: 0.5									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td><input type="checkbox"/> (a) Human subjects</td> <td><input type="checkbox"/> (b) Human tissues</td> <td><input checked="" type="checkbox"/> (c) Neither</td> </tr> <tr> <td><input type="checkbox"/> (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td><input type="checkbox"/> (a2) Interviews</td> <td colspan="2">This is a non-clinical IIDEA project.</td> </tr> </table>			<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither	<input type="checkbox"/> (a1) Minors			<input type="checkbox"/> (a2) Interviews	This is a non-clinical IIDEA project.	
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither									
<input type="checkbox"/> (a1) Minors											
<input type="checkbox"/> (a2) Interviews	This is a non-clinical IIDEA project.										
SUMMARY OF WORK <i>(Use standard unredacted type. Do not exceed the space provided.)</i> <p>The purpose of this project is to study various aspects of genome structure and function of the Aleutian mink disease parvovirus (ADV).</p> <p>A major effort is to determine which segments of the ADV genome govern pathogenicity and the ability of various virus isolates to replicate permissively in cell culture. Using a series of segmental exchanges between a full-length infectious molecular clone of the nonpathogenic ADV-G strain and a partial (15 - 88 map unit) clone of pathogenic ADVs, we have found that clones containing the 53-65 mu segment of pathogenic ADVs express viral antigen in cell culture but do not produce infectious virus in cell culture. This segment is part of the coding region for the capsid proteins and only 4 amino acid differences occur between ADV-G and the pathogenic ADVs; this segment does not contain the hypervariable region. Using a mutagenesis strategy based on the polymerase chain reaction we have mutated one of these 4 amino acids and found that virus rescued from this clone retains the characteristics of the parent ADV-G.</p> <p>Several of the chimeric clones yielded ADV that could be rescued in cell culture and these viruses were assayed for the ability to induce disease in adult or newborn mink. No virus that replicated in cell culture caused any significant disease in either adult or newborn mink.</p> <p>We have continued to study the expression of ADV proteins in eukaryotic expression systems. We have found that synthesis of empty ADV particles was directed by a recombinant vaccinia virus that contained the coding sequences for both ADV capsid proteins (VV-IL1). These particles could be purified and banded at a density of ca. 1.33 g/ml in cesium chloride, identical to empty ADV particles produced in standard ADV infections. VV-IL1 was injected into mice; 8/8 mice produced detectable antibody as assayed by indirect immunofluorescence, but only 2/8 produced neutralizing antibody. Vaccination of adult mink with either VV-IL1 or WR vaccinia virus led to an accelerated form of ADV when the mink were challenged with a low dose of ADV.</p>											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 A1 00265-10 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. E. Race Research Veterinarian LPVD, NIAID

Other: B. Caughey Staff Fellow LPVD, NIAID

K. Neary Staff Fellow LPVD, NIAID

B. Chesebro Chief LPVD, NIAID

COOPERATING UNITS (if any)

Dr. A. Haase, Chief, Dept. Microbiology, University of Minnesota, Minneapolis, MN; Dr. Al Jenny, U.S.D.A., Ames, IA

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

2.2

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Scrapie is a spongiform encephalopathy which under natural conditions affects sheep and goats. Similar diseases have been described in mink, mule deer, elk, and most recently, cattle. The disease in cattle has reached epidemic proportions within Great Britain's dairy herds. At least three human diseases, Creutzfeldt-Jakob disease, Kuru, and Gerstmann-Sträussler syndrome, are histologically indistinguishable from scrapie. Available evidence suggests that all of these diseases are caused by similar transmissible agents. The infectious agents which cause these diseases are particularly interesting because no nucleic acid genome has been associated with them. Preparations that contain large amounts of scrapie infectivity do contain an aggregated proteinase K (PK)-resistant form of an endogenous protein, designated PrP. The PK-resistant form of this protein (PrP-res) is specific for diseases of the spongiform encephalopathy group. Furthermore, some experimental evidence suggests that PrP-res is important in disease pathogenesis. However, considerable controversy surrounds the exact relationship between PrP-res and the infectious agent of scrapie. A few investigators believe PrP-res is itself the scrapie agent. Alternatively, it's possible that the protein could be a component of the agent or merely accumulate as a byproduct of disease. Regardless of the relationship, detection of PrP-res offers an important tool for the study of the spongiform encephalopathies.

We have utilized scrapie-infected cell cultures which we developed to study the scrapie agent and PrP. PrP exists in two forms, PrP-sen which is found in both normal and scrapie-infected animals and is easily destroyed by exposure to PK, and PrP-res which is found only in scrapie-affected animals and is partially resistant to destruction by PK. We have shown that a close association exists between the detection of PrP-res by immunoblotting and the presence of the scrapie agent. Due to the recent outbreak of bovine spongiform encephalopathy and the potential risk that infected cattle might have for humans, we have initiated studies which could lead to a more rapid method for diagnosing spongiform encephalopathies. Other studies deal with the influence of the PrP gene on scrapie incubation period and susceptibility, mechanisms that might explain species tropism and studies seeking to determine factors which account for the conversion of PrP-sen to PrP-res. We are also trying to identify better methods for inactivating high titered scrapie-infected tissues and surfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00266-09 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. H. Evans Chemist LPVD, NIAID

Other: R. J. Monk Senior Staff Fellow LPVD, NIAID

COOPERATING UNITS (if any)

Dr. Seth Pincus, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton, MT

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Many retroviruses undergo genetic alterations *in vivo* which may affect the viral oncogenicity, cellular tropism, or immune elimination. For example the transforming avian and murine retroviruses have acquired genetic sequences of the host which are directly oncogenic. Murine leukemia viruses (MuLVs) readily undergo recombination with mouse genomic sequences to generate variants which exhibit an altered host range of infectivity and can activate cellular oncogenic genes. Point mutation of lentiviruses such as the equine infectious anemia virus (EIAV) or the human immunodeficiency virus (HIV) may contribute to the generation of variants which escape the host immune response. The major emphasis of this project is the occurrence and mechanisms of genetic alteration in retroviruses and the consequences of such alterations. In previous studies we have examined the tissue-specific expression of host range variants which are generated after inoculation of an MuLV. Since the generation of such variants results in a mixed infection of viruses with different host ranges, there is a potential for viral pseudotyping to occur in which the genome of one virus is encapsulated in the virion coat of another. We have found a distinct pattern of pseudotyping which correlates with the onset of leukemia in mice infected with the lymphocytic leukemia virus, M-MuLV. In both the spleen and thymus, host range variants are completely pseudotyped by the M-MuLV during most of the preleukemic phase of disease. Thus, all virions exhibit the host range of M-MuLV, regardless of which viral genome they contain. Complete pseudotyping persists in the spleen throughout the course of infection. However, in the thymus a high proportion of virions which exhibit the variant host range arise immediately preceding the earliest onset of leukemia. Further studies will focus on the relationship of this burst of variant virions to malignant transformation.

Studies on the point mutation rate of retroviruses have determined the rate of viable progeny viruses to be approximately 20-fold slower than previous reports. This study is currently being extended to determine the *in vivo* polymerase error rate for an MuLV. Several proviruses which have undergone a single replication cycle have been molecularly cloned into a vector which allows both direct DNA sequencing as well as *in vitro* transcription and RNA fingerprinting. Analysis of these clones will yield the first *in vivo* polymerase error rate determination for a eukaryotic virus.

In previous studies numerous monoclonal antibodies (mAb) directed at MuLV antigens have been developed. We have initiated collaborative experiments with Dr. Pincus of the LMSF to construct and test ricin A-chain immunotoxins (IT) for their ability to selectively kill cells infected by MuLVs. Several immunotoxins exhibit cytotoxic activity for infected cells. These include an immunotoxin which exhibits significant cytotoxicity of cells infected with all classes of MuLVs and another immunotoxin which exhibits virtually complete killing of cells infected with a highly neurovirulent MuLV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00468-06 LPVD
PERIOD COVERED October 1, 1990 to September 30, 1991		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Biology of Human AIDS Retrovirus		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</i> PI: B. Chesebro Chief LPVD, NIAID Other: W. Maury Staff Fellow LPVD, NIAID		
COOPERATING UNITS <i>(if any)</i> Julie Metcalf, NIAID, Bethesda, MD; Diane Griffin, Johns Hopkins University School of Medicine, Baltimore, MD.		
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 0.4	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews This is a non-clinical AIDS-related project.		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided..)</i> <p> This project is aimed at studying cell tropism of HIV infectivity <i>in vitro</i>. Initially we developed a focal infectivity assay using plastic-adherent CD4-positive HeLa cells as targets for HIV infection <i>in vitro</i>. Subsequently this approach was used to study human glioma and squamous cell carcinoma cell lines expressing the same CD4 vector as the HeLa cells. Surprisingly these other cell lines could not be infected directly by HIV. This block in infectivity was found to be at the point of viral entry because the cells produced infectious HIV after transfection with viral DNA or infection with HIV pseudotyped by amphotropic murine leukemia virus. These results indicate that other cellular molecules in addition to CD4 are required for HIV fusion and entry into target cells. </p> <p> Additional work has been done using a new clone of CD4-positive HeLa cells to assay expression of infectious HIV in blood cells of seropositive human patients. The results indicated that HIV infectivity was expressed at very low levels, up to a maximum of 1 in 10⁴ peripheral blood mononuclear cells. The new clone of HeLa cells was 300-fold more sensitive to infection than previous clones, and HIV isolates from 95% patients could be detected in these cells. However, virus from 5% of patients did not infect the CD4-positive HeLa cells. Current work on these isolates suggests that they are probably macrophage-tropic virus strains. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00524-04 LPVD												
PERIOD COVERED October 1, 1990 to September 30, 1991														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Factors in Susceptibility and Resistance to Rabies Virus in Mice														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: L. L. Perry</td> <td style="width: 30%;">Expert</td> <td style="width: 30%;">LPVD, NIAID</td> </tr> <tr> <td>Other: D. Lodmell</td> <td>Staff Scientist</td> <td>LPVD, NIAID</td> </tr> <tr> <td>T. Ullrich</td> <td>Staff Scientist</td> <td>Ribi Laboratories, Hamilton, MT</td> </tr> <tr> <td>F. Hayes</td> <td>Research Technician</td> <td>LVP, NIAID</td> </tr> </table>			PI: L. L. Perry	Expert	LPVD, NIAID	Other: D. Lodmell	Staff Scientist	LPVD, NIAID	T. Ullrich	Staff Scientist	Ribi Laboratories, Hamilton, MT	F. Hayes	Research Technician	LVP, NIAID
PI: L. L. Perry	Expert	LPVD, NIAID												
Other: D. Lodmell	Staff Scientist	LPVD, NIAID												
T. Ullrich	Staff Scientist	Ribi Laboratories, Hamilton, MT												
F. Hayes	Research Technician	LVP, NIAID												
COOPERATING UNITS (if any) None														
LAB/BRANCH Laboratory of Persistent Viral Diseases, Hamilton, MT 59840														
SECTION														
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892														
TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Monoclonal antibody-mediated depletion of CD4+ Th and CD8+ Tc subsets in street rabies virus resistant strains of mice was used to determine the role of each subset in host immunity. Depletion of CD4+ Th was associated with a complete loss of resistance in both SJL/J and BALB/cByJ mice, while depletion of CD8+ Tc was without effect in either strain. Animals lacking CD4+ T cells as a result of treatment also failed to mount an IgG neutralizing response to rabies viral antigens, although IgM responses were intact. This data support previous findings on the protective role of IgG but not IgM neutralization responses in rabies disease. The data also argue against a role for cytotoxic T cells in the prevention or elimination of rabies virus infection in the central nervous system, since intact or CD8 depleted BALB/cByJ mice retain the capacity to clear a transient viral infection of the CNS. These findings were published in the Journal of Virology. </p> <p> Studies were also initiated on the role of macrophages in host resistance to street rabies virus. Susceptible strains of mice such as A/WySn or A.SW express a number of defects in macrophage responsiveness to IFN that have been related to host susceptibility to bacterial disease. A strain mice are also deficient in production of C5, and thus in release of the C5a macrophage chemotactic factor. Experiments to compare the peritoneal inflammatory responses of susceptible A.SW and resistant SJL/J mice revealed that A.SW mice are unable to mount a rapid and effective macrophage inflammatory response after intraperitoneal infection with street rabies virus. These animals can be protected, however, by pretreatment with an adjuvant containing trehalose dimycolate developed by Ribi Laboratories. Adjuvant pretreatment results in a 5 to 7 fold increase in the number of inflammatory cells present in the peritoneum at the time of infection. Experiments are currently underway to define any qualitative differences that may exist in macrophage activity of susceptible and resistant hosts. Results will be submitted for publication. </p>														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00580-02 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Scrapie Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Caughey, Ph.D. Senior Staff Fellow LPVD

Other: Richard Race, D.V.M. Research Veterinarian LPVD

Bruce Chesebro, M.D. Chief LPVD

Kholari Bhat, Ph.D. Senior Staff Fellow LMSF

COOPERATING UNITS (if any)

Drs. A. Dong and W. S. Caughey, Dept. of Biochemistry, Colorado State University, Ft. Collins, Colorado.

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.9

PROFESSIONAL:

1.0

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

This is a non-clinical IIDEA project.

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Scrapie and related transmissible spongiform encephalopathies result in the accumulation of an abnormal protease-resistant form of an endogenous brain protein called PrP. This protease-resistant PrP (PrP-res) can aggregate into fibrils and form amyloid-like plaques. There is substantial evidence that the formation of PrP-res is scrapie-specific and important in the pathogenesis of the disease. However, it is not yet clear whether PrP-res is the transmissible agent itself, a component of the agent, or a byproduct of the infection which happens to co-fractionate with scrapie infectivity.

Since the replication or pathogenesis of the scrapie agent may involve the production of PrP-res, we have continued studies of the properties and biosynthesis of both PrP-res and the normal, protease-sensitive form of PrP in scrapie-infected neuroblastoma cells. In the last year, we have determined that PrP-res is synthesized much more slowly than the normal PrP isoform, and thus may be generated from it. This was supported by experiments showing that PrP-res is made from a precursor that, like the normal isoform, resides on the cell surface in a phospholipase- and protease-sensitive state. Once it is made, PrP-res is truncated at the N-terminus by lysosomal proteases, and therefore must be translocated to the lysosomes. When considered together, these findings imply that the conversion of PrP to the protease-resistant state is a post-translational event that occurs at the cell surface or along the endocytic pathway to the lysosomes.

The molecular nature of the conversion of PrP to a protease-resistant form remains a mystery. It is possible that a conformational abnormality accounts for the characteristics of PrP-res. To investigate this possibility, we have used a recently developed Fourier transform infrared spectroscopy technique to analyze the secondary structure of the proteinase K-resistant core of PrP-res as it exists in highly infectious fibril preparations. The analysis indicated that PrP-res is composed primarily of β -sheet (47%), which is consistent with its amyloid-like properties. In addition, significant amounts of turn and α -helix were identified, indicating that amyloid fibrils need not be exclusively β -sheet. The infrared-based secondary structure compositions were then used as constraints to improve the theoretical localization of the secondary structures within the PrP-res molecule. We are now developing a method of purifying the normal PrP isoform under nondenaturing conditions so that we will be able to compare its conformation to that of PrP-res.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00610-01 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of H-2 I-E in recovery from Friend virus induced leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. L. Perry Expert LPVD, NIAID

Other: M. Miyazawa Visiting Fellow LPVD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.40

PROFESSIONAL:

0.40

OTHER:

0.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous experiments defining the H-2 restriction element specificity of T cells responsive to F-MuLV envelope glycoproteins revealed that mAbs directed against I-A^b and, to a lesser extent, I-E^k inhibit in vitro proliferative responses. To further analyze the possible role of H-2 I-E gene products in immunity to Friend virus induced leukemia, animals infected with the Friend virus complex (F-MuLV and spleen focus forming virus) were treated in vivo with mAbs directed against the alpha or beta chain of the I-E surface glycoprotein. Administration of anti-I-E^k_α or anti-I-E^k_β completely inhibited recovery from splenomegaly in (A.BY x B10.A)F₁ mice, while treatment with anti-I-A^b was virtually without effect. Treatment with anti-I-E mAb is associated with complete suppression of the IgG neutralization response with no depression of neutralizing IgM titers. Anti-I-E mAb treated animals also display a reduced capacity to transfer protection to naive, virus infected recipients, indicating a paucity of Friend virus reactive cells in the spleen. I-E restricted T cells are specific for epitopes on the virus envelope rather than on core proteins as determined by inhibition of delayed type hypersensitivity responses in vaccinia-env versus vaccinia-gag immunized hosts. The mechanism of inhibition does not appear to involve active T cell suppression, however, as evidenced by the absence of any effects on recovery or DTH in immune recipients of splenocytes from treated donors. A role for I-E in recovery was supported by additional studies in highly resistant, I-E- (A.BY x B10)F₁ mice expressing or lacking an I-E^k_α transgene. Animals expressing the transgene (which allows for surface expression of an I-E gene product through complementation with an endogenous E_β gene) consistently exhibit a 30% higher incidence of persistent splenomegaly and a 15% higher incidence of mortality as compared to transgene negative litter mates. A manuscript is currently in preparation to describe this observation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00611-01 LPVD

PERIOD COVERED

October 1, 1990 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enrichment of Hematopoietic Stem Cells from Mouse Bone Marrow

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G.J. Spangrude

Senior Staff Fellow

LPVD, NIAID

Other:

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

1.0

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to investigate the early stages of hematopoiesis in a mouse model system, utilizing methodology for obtaining highly enriched populations of hematopoietic stem cells (HSC) from mouse bone marrow tissue. These cells are then transplanted into irradiated recipient animals in a congenic strain combination which allows identification and quantitation of graft-derived cells over the lifetime of the transplant recipients. Using this approach, one can ask a variety of questions regarding the nature of HSC in the mouse, including the identification of surface antigens expressed by these cells, the functional heterogeneity of the stem cell compartment at a single cell level, and the susceptibility of HSC populations to infection by pathogenic mouse viruses and by retroviral constructs which may be exploited as vectors to introduce new genetic material into the stem cell compartment. Further, in vitro culture systems and growth factors can be tested for the ability to propagate and perhaps influence self-renewal of the enriched HSC populations. In order to realize these goals, it is critical to develop confidence in the methodology utilized to enrich the HSC population. In particular, the enrichment method should select for all primitive HSC present in the bone marrow, and not simply a subpopulation which may or may not include the most primitive members of the HSC compartment. This possibility has been considered in some detail, starting from the hypothesis that mouse HSC can be identified by immunofluorescent staining as those cells which express the Ly6A/E and Thy-1 antigens in the absence of antigens characteristic of differentiated lineages of blood cells (Lineage negative). Surprisingly, while mouse HSC seem largely to fit the phenotypic description of Ly6A/E positive and Lineage negative, in mouse strains expressing the Thy-1.2 allele a significant level of HSC activity was demonstrated within both Thy-1 positive and Thy-1 negative populations. In contrast, the Thy-1.1 allele was expressed by the majority of HSC. Therefore, some variability exists in the cell surface antigenic phenotype of mouse HSC.

LABORATORY OF VECTORS AND PATHOGENS
Rocky Mountain Laboratories
Hamilton, Montana
1991 Annual Report
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ANNUAL REPORT
LABORATORY OF VECTORS AND PATHOGENS
HAMILTON, MONTANA
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1990, TO SEPTEMBER 30, 1991

Members of the Laboratory of Vectors and Pathogens have continued their molecular dissection of important human pathogens during the past year. This multidisciplinary approach to microbial pathogenesis combines the fields of biochemistry, immunology, electron microscopy, medical entomology and molecular biology into a cohesive unit with strong interaction among its own members and with scientific units both inside and outside of the NIH system. This interaction together with an active seminar and guest researcher program insures that the best experimental approaches available are rapidly incorporated into the research programs of the laboratory. Since Lyme borreliosis is now the most common arthropod-borne disease in the United States, the Laboratory has made a major commitment of resources to the development of improved diagnostics and to a detailed understanding of how *Borrelia burgdorferi* produces its long list of both acute and chronic clinical features. The Laboratory of Vectors and Pathogens functions in three major experimental groups. Dr. Claude F. Garon serves as Laboratory Chief.

The Arthropod-borne Diseases Section, under the direction of Dr. Tom G. Schwan, is involved in numerous investigations relating to the molecular biology, infectivity, pathogenicity, antigenicity, and immunogenicity of *B. burgdorferi*. Efforts to improve the serodiagnosis of Lyme disease have been encouraging through the cloning and expression of a 39 kDa antigen of *B. burgdorferi* that is specific to this species of spirochete. To confirm that anti-P39 antibodies are produced consistently in animals exposed to infectious spirochetes, white-footed mice and laboratory mice, *Mus musculus*, were experimentally inoculated with either infectious or noninfectious *B. burgdorferi*. Antibodies to the P39 antigen were then determined by immunoblot analysis at 21 days postinoculation. All mice inoculated with infectious *B. burgdorferi* produced anti-P39 antibodies and produced positive cultures of spirochetes when triturations of their urinary bladder and spleen were put in BSK-II culture media. Mice inoculated with similar numbers of inactivated or viable noninfectious spirochetes did not have detectable levels of anti-P39 antibodies. Mice infected with *B. burgdorferi* following exposure to ticks, *I. dammini*, produced anti-P39 antibodies within 3-7 days postinfection, indicating that the P39 antigen is an effective immunogen in natural infections. Also, antibodies to P39 were the predominant antibodies early in infection. The results demonstrate that anti-P39 antibodies are produced only in response to an active infection and are therefore reliable markers for infection in both experimentally and naturally infected animals. Serological kits containing the recombinant P39 antigen are under current review by the Food and Drug Administration and should be available within the next year. Members of the Arthropod-borne Diseases Section have identified another unique, species-specific protein of *B. burgdorferi*. This protein, P22-A, is conserved among all the North American and European isolates that were examined. The gene encoding this antigen was cloned and the recombinant was used to screen sera collected from experimentally infected white-footed mice. Although antibodies were detected in all infected mice at 21 days postinoculation, the responses were stronger in other mice that were inoculated with inactivated and lysed bacteria. These observations and immune electron microscopy analyses, suggested that P22-A is concentrated in the periplasmic space. P22-A also exhibited size heterogeneity among different isolates of the spirochete and varied in estimated size between 20 and 23 kDa. As a group, however, these proteins retained antigenic homogeneity and may prove useful in serological assays using a mixture of recombinant antigens specific to the Lyme disease spirochete. Preliminary studies exploring *in vivo* antigenic variation of *B. burgdorferi* in mice have been completed. Adult laboratory mice were inoculated intraperitoneally with a low-passaged culture of an uncloned strain of *B. burgdorferi* and 16 months later spirochetes were reisolated from the urinary bladder of 94% of the mice. Spirochetes recovered from the urinary bladder of one persistently infected mouse were tested for infectivity and found to be infectious when passaged into four laboratory mice. Western blot analysis of immune serum from each of the persistently infected mice demonstrated that spirochetes used to infect the mice reacted differently when compared with the spirochetes

subsequently reisolated from the mice, demonstrating for the first time that changes in antigenic reactivity had occurred in the spirochete populations during persistent infection (Schwan, Simpson, Gage).

Also participating in research on the molecular biology of *Borrelia* is the Structural Pathobiology Section, under the direction of Dr. Claude F. Garon. The immunological interactions between the Lyme disease spirochete, *Borrelia burgdorferi*, and its mammalian hosts are poorly understood. Although most mammalian hosts mount an antibody response to the spirochete, the antibodies are often serologically cross-reactive with other spirochetes, and seronegative individuals with active infections have been encountered by standard screening criteria. Furthermore, strain variation among *B. burgdorferi* isolates and antigenic variation within populations render immunodiagnostics based on monoclonal antibodies insensitive and unreliable for detection of circulating and excreted antigens in some hosts. Therefore, clinical symptoms, patient history, and occasional primary isolations of the spirochete from blood or tissue biopsy specimens provide the bases for most diagnoses. Such problems are often cited as factors influencing the reportedly poor diagnostic acumen for Lyme disease. Considerable work is directed toward identifying conserved, species-specific cell surface antigens for diagnostic use and epidemiological and pathogenic studies. Expression of outer surface protein A (Osp A) is considered universal among *B. burgdorferi* isolates, but it is not present among related species. Surface-exposed immunodeterminants on Osp A appear to be antigenically variable, since no surface-reactive monoclonal antibodies to Osp A have been reported to bind to all of the strains tested. Similarly, Osp B is apparently unique to *B. burgdorferi*; however, the expression of Osp B is reportedly variable in some strains. Recent experiments have shown that nuclease-protected DNAs are exported from *B. burgdorferi* cells in association with membrane vesicles. Indirect evidence suggests that these vesicles may be produced by spirochetes in vivo, providing a sustained antigenic challenge to hosts that maintain a limited population of spirochetes. To determine whether *B. burgdorferi* vesicles occur in experimentally infected mice, polyclonal rabbit sera were generated against vesicles and against a prominent, vesicle-associated protein and with an apparent mass of 83 kDa. Using these reagents, an immune electron microscopic assay for first capturing and then identifying extracellular *B. burgdorferi* antigens was developed. The study was extended to include antigen detections in *Ixodes* ticks and in mouse, dog, and human samples. This approach has proven effective for demonstrating *B. burgdorferi* infections, and should enable further ultrastructural examination of the effects of these bioproducts on hosts. The use of rRNA sequences as chronometers of evolutionary divergence is now widespread and its advantage over classical taxonomic approaches is well documented. To date, over 500 small subunit rRNA sequences have been determined and used for this purpose. Section members have determined in near entirety, the 16S rRNA sequences of several borrelial species and isolates. The phylogenetic relationships among the isolates were determined through calculations of sequence similarity and Knuc values and the data presented as an unrooted phylogenetic tree. In addition, rDNA restriction patterns were determined using Southern blot analyses, and the data compared with published genomic grouping schemes. Utilizing the sequences determined here, members of the Section have in addition investigated potential secondary and tertiary structural elements and sought to identify aspects of the sequence and its corresponding structure which may be unique to *Borrelia*. Through the use of sequence alignment programs and the comparative sequence approach, a secondary structure map for the *Borrelia* 16S rRNA molecule has been constructed. An analysis of the *Borrelia* tetra-loops was also conducted and the sequences were compared with those from other organisms. Chloro (2,2':6,2"-terpyridine) platinum (II) chloride is a commercially available compound that shows effective intercalative binding to nucleic acid molecules. And by virtue of its platinum moiety, offers the opportunity for efficient backscattered electron detection and ultrastructural localization of nucleic acid containing compartments in cells. Binding is relatively specific, stable, and spectrophotometrically detectable. Platinum (II) chloride appears to be an effective and useful stain for nuclei and nucleoli expanding the utility of backscattered electron detection in scanning electron microscopy (Garon, Dorward, Marconi).

The Molecular Pathogenesis Unit under the direction of Dr. Witold Cieplak, Jr., has made important new observations on the biology and molecular biology of human pathogens. During the past year, the Unit

has created and characterized a number of interesting mutant analog forms of the enzymatically active A subunit of the *E. coli* heat-labile enterotoxin. This toxin is highly similar in both primary sequence and overall structure to cholera toxin. As such, the results of these studies are likely to be applicable to structural biology of cholera toxin. They have been able to produce the A subunit alone as a recombinant fusion protein and determined that its enzymatic properties are qualitatively indistinguishable from the authentic toxin. However, detailed studies of the isolated A subunit have revealed an interesting feature of its activity requirements. It had been predicted, based on conventional biochemical studies of cholera toxin, that limited proteolytic cleavage and reduction were required to express maximal enzymatic activity. Their studies, which represent the first investigations of the isolated A subunit (recombinant or otherwise) indicate that this is not the case when the A subunit alone is examined. Accordingly, it appears that the requirement for proteolytic cleavage and reduction applies only to the holotoxin and serves to free the active amino-terminal portion of the A subunit from the remainder of the toxin (B oligomer) thereby relieving apparent steric constraints on the enzyme. This represents a novel finding and alters our concept of the molecular mechanisms associated with translocation of the toxin into cells and activity requirements. Continued studies of the site-directed mutant forms of *E. coli* heat-labile toxin A subunit have identified at least two areas that are critical for enzymatic activity. Alterations in the amino terminal region of the A subunit, which bears sequence similarity to other ADP-ribosylating toxins, completely eliminate ADP-ribosyltransferase activity. However, further analyses showed that mutations in this region also significantly affect the overall conformation of the subunit. In contrast, mutagenic substitutions at glutamic acids 110 or 112 severely reduce enzyme activity (greater than a factor of 100) but do not appear to have global conformational effects. Therefore, the glutamic acid at either position 110 or 112 appears to represent the equivalent of catalytically important glutamic acids that have been identified in diphtheria toxin, *Pseudomonas* exotoxin A and pertussis toxin. Accordingly, these glutamic acid residues in heat-labile toxin and the corresponding residues in cholera toxin, and not the amino-terminal region, are likely to serve as suitable targets for the production of mutant holotoxoids for potential vaccine use. During the past year, they have developed more accurate and revealing assays for determining the virulence mechanisms associated with *C. jejuni*. Prior studies, using laboratory adapted strains of *C. jejuni*, appeared to indicate that *C. jejuni* were not equipped to survive within either professional or non-professional phagocytic cells of human origin. However, within the past year they have obtained fresh clinical isolates of *C. jejuni* that have been passaged only a few times and appear to possess the ability to survive and replicate within cultured epithelial cells. The intracellular replication of *C. jejuni* is followed by an overt cytotoxic effect on the target cells caused by a yet undefined factor or metabolite but may indicate the presence of a cytotoxin. The ultrastructural and physiologic features of these interactions of have been examined some detail. The internalization process bears close resemblance to phagocytosis as judged by electron microscopic observations. Organisms are enveloped by microvillar processes and reside in vacuoles in the intracellular compartments. Internalization is inhibited by agents which disrupt microfilament formation (e.g. cytochalasins B and D) but not by compounds which inhibit microtubule formation (e.g. colchicine). They have also found that *C. jejuni* is capable of penetrating differentiated and polarized epithelial cells (Caco-2) in a fashion similar to that of other known invasive pathogens (e.g. *Salmonella*, *Yersinia*). Finally, they have found that the growth temperature of the organism has a significant effect on the internalization process. Specifically, *C. jejuni* grown at 37°C are internalized and/or survive significantly better than organisms grown at either 30°C or 42°C. This finding may contribute to an explanation for the apparent commensal nature of *C. jejuni* in birds where the core temperature approximates 42°C. Additionally, altered profiles of surface proteins have been detected among organisms grown at these various temperatures (Cieplak, Konkel).

The focus of the Laboratory of Vectors and Pathogens has remained during the past year on the use of modern methods of molecular biology to define, in molecular terms, critical aspects of the host-pathogen relationship.

ADMINISTRATIVE

Drs. Willy Burgdorfer and John Munoz worked as scientist emeritus members of the laboratory during the year providing valuable service and support.

Guest Researchers in the Laboratory of Vectors and Pathogens have included Dr. Stanley Falkow (Stanford University School of Medicine), Dr. Lucy Tompkins (Stanford University), Dr. Olivier Peter (Institut Central des Hopitaux Valaisans, Switzerland), Dr. Willard Granath (Division of Biological Sciences, University of Montana), Dr. William Whitmire (National Animal Disease Center, Ames, Iowa).

Visitor/Collaborators who spent varying amounts of time interacting with members of the LVP scientific staff included:

<u>Name</u>	<u>Affiliation</u>
Dr. Richard Blakemore	University of New Hampshire
Dr. Christopher Grant	Stanford University
Dr. Telsa Mittelmeier	University of Arizona
Dr. Jorge Giron	Stanford University
Dr. John Halperin	State University of New York at Stony Brook

Joining the laboratory as an IRTA Fellow during the year was Dr. D. Scott Samuels (University of Arizona). Completing a Visiting Associateship was Dr. Warren Simpson.

The Laboratory hosted the Second Annual Meeting of Grantees Studying the Pathogenesis of Lyme Borreliosis in September.

HONORS AND AWARDS

Dr. Claude F. Garon

Served as Chairman, State Board of Directors, Center of Excellence in Biotechnology, Montana Science and Technology Alliance
Faculty Affiliate - Division of Biological Sciences, University of Montana
Internal Advisory Committee, University of Montana Electron Microscopy Facility

Invited speaker

Department of Microbiology, Louisiana State University
Louisiana Society for Electron Microscopy 30th Anniversary Meeting, New Orleans, LA
Hamilton Lions Club, Hamilton, MT
Meeting of Grantees Studying the Pathogenesis of Lyme Borreliosis, Bethesda, MD
Scanning 91, Atlantic City, NJ
Lyme Disease Breakthroughs + Implications for Patient Management - Lyme Borreliosis Foundation, Santa Monica, CA
Molecular Biology of Spirochetes, Annecy, France
Second Annual Meeting of Grantees Studying the Pathogenesis of Lyme Borreliosis, Hamilton, MT

Ad Hoc Reviewer for NIAID-Microbiology and Infectious Diseases Research Committee

Served on Special Review Committee, Microbiology and Immunology - NIAID
Participated in Program review of the Centers for Disease Control's Division of Vector-borne Infectious Diseases.

Reviewed manuscripts

Infection and Immunity

Dr. Tom G. Schwan

Invited speaker

NIAID/NIAMS workshops for grantees as the pathogenesis of Lyme disease, Bethesda, MD.

Lyme Disease Symposium and Workshop, Laboratory Center for Disease Control, Health and Welfare, Guelph, Ontario, Canada.

State of the Art Conference on the Diagnosis of Treatment of Lyme Disease, NIAID/NIAMS, Bethesda, MD.

Lyme Borreliosis Foundation Meeting, Santa Monica, CA.

Presentor and invited chairman for session on Lyme Disease, Annual Meeting of the American Society for Microbiology, Dallas, TX.

Invited Participant for NIAID/NIAMS workshops on Lyme disease, Hamilton, MT.

COMMITTEES

Ad Hoc reviewer for MIDRC, NIAID, Bethesda, MD

Ad Hoc reviewer for Lyme Borreliosis Foundation

Elected to the Executive Council of the American Committee of Medical Entomology

Reviewed manuscripts for

American Journal of Tropical Medicine and Hygiene (4)

Experimental Parasitology (1)

Journal of Clinical Investigation (2)

Journal of Infectious Diseases (2)

Journal of Medical Entomology (3)

Journal of Wildlife Diseases (1)

Infection and Immunity (1)

Proceedings of the National Academy of Sciences (1)

Dr. Witold Cleplak, Jr.

Invited speaker

NIH *Campylobacter* Symposium, Monterrey, CA

Reviewed manuscripts for

Infection and Immunity

Vaccine Research

Dr. Willy Burgdorfer

Invited to present lecture at Lyme Disease Symposium Mid-Hudson Civic Center, Poughkeepsie, New York.

Received Walter Reed Medal by the American Society of Tropical Medicine and Hygiene, 39th Annual Meeting, New Orleans, LA

Invited to present lecture "From erythema migrans to Lyme disease: who, when, and what," Midwest Lyme Disease Symposium, Lincoln, Nebraska

"Disease Detectives" - Reference to Dr. Burgdorfer's discovery of the Lyme disease spirochete in National Geographic, vol 179, January 1991.

Awarded Honorary Doctor of Science Degree by the University of Marseille, France

Invited to present video tape lecture "Lyme borreliosis: 10 years after discovery of the etiologic agent," Graz, Austria.

Presented by videotape lecture "Lyme Borreliose - Uebersicht an Forschung eines Jahrzehnts," 2nd Internationale Tagung Infektionskrankheiten in der Alpenlandern," Seis am Schlern, Italy

Presented lecture on Lyme Disease at the Community Medical Center Foundation's annual meeting, Missoula, MT.

Invited to lecture at Lyme Borreliosis Foundation's Conference, Lyme Borreliosis Breakthroughs and Implications for Patient Management, Los Angeles, California

Invited to speak at Ravalli County Museum, Hamilton, MT

Served as instructor at 41st Acarology Summer Program, Ohio State University, Columbus, OH

Invited to present lecture "Ten years after the discovery of *Borrelia burgdorferi*," Ohio Department of Health, and Columbus Health Department, Columbus, OH.

Served as Co-Editor of "Erythema Migrans (Lyme) Borreliosis in Europe; 29 chapters, Springer Verlag (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00480-06 LVP
PERIOD COVERED October 1, 1990, to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathogen-arthropod interactions of Vector-borne Diseases Affecting Public Health		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Tom G. Schwan Sr. Staff Fellow LVP, NIAID OTHERS: K. L. Gage IRTA Fellow LVP, NIAID R. H. Karstens Bio Lab Tech (Micro) LVP, NIAID		
COOPERATING UNITS (if any) Microbial Diseases Laboratory, California Department of Health, Berkeley (Jane Wong); Texas Department of Health, Austin (Julie Rawlings, Glenna Teltow)		
LAB/BRANCH Laboratory of Vectors and Pathogens		
SECTION Arthropod-borne Diseases		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <p>The objectives of this project are to 1) use both molecular and classical approaches to investigate pathogen-arthropod interactions of vector-borne diseases of human importance in the United States, and 2) examine basic biological questions concerning the behavior of ticks and fleas that influence their ability to transmit infectious agents. In this project, we have already 1) developed DNA hybridization probes to detect <i>Yersinia pestis</i>, the causative agent of plague, in experimentally infected fleas and rodents, and 2) developed a recombinant vaccine that protected mice when challenged with virulent <i>Y. pestis</i>. More recently we examined the ability of soft ticks to become infected with <i>Y. pestis</i> and in some cases, maintain the infection for up to one year, demonstrating the potential for some species of soft ticks to maintain and reservoir this bacterium in nature. A final project with <i>Y. pestis</i> is currently underway to use the polymerase chain reaction (PCR) to detect <i>Y. pestis</i> in <i>Xenopsylla cheopis</i>, the classical flea vector of urban plague, which we have colonized at RML.</p> <p>We have recently completed the description of a new species of <i>Argas</i> tick from California and determined its life cycle and potential as a vector to transmit an <i>Orbivirus</i> (Reoviridae) to humans.</p> <p>Most of our current and future efforts will focus on novel isolates of relapsing fever <i>Borrelia</i> from human patients in the western United States and explore their molecular basis for antigenic variation.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00488-05 LVP																							
PERIOD COVERED October 1, 1990, to September 30, 1991																									
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Ultrastructural Analysis of Antigenic Determinants in Pathogens																									
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Claude F. Garon</td> <td style="width: 30%;">Chief</td> <td style="width: 20%;">LVP, NIAID</td> </tr> <tr> <td rowspan="6">OTHERS:</td> <td>S. F. Hayes</td> <td>Bio Lab Tech (Micro)</td> <td>LVP, NIAID</td> </tr> <tr> <td>D. Corwin</td> <td>Bio Lab Tech (Micro)</td> <td>LVP, NIAID</td> </tr> <tr> <td>W. Burgdorfer</td> <td>Scientist Emeritus</td> <td>LVP, NIAID</td> </tr> <tr> <td>D. W. Dorward</td> <td>Staff Fellow</td> <td>LVP, NIAID</td> </tr> <tr> <td>L. L. Lubke</td> <td>Biologist</td> <td>LVP, NIAID</td> </tr> <tr> <td>T. G. Schwan</td> <td>Sr. Staff Fellow</td> <td>LVP, NIAID</td> </tr> </table>			PI:	Claude F. Garon	Chief	LVP, NIAID	OTHERS:	S. F. Hayes	Bio Lab Tech (Micro)	LVP, NIAID	D. Corwin	Bio Lab Tech (Micro)	LVP, NIAID	W. Burgdorfer	Scientist Emeritus	LVP, NIAID	D. W. Dorward	Staff Fellow	LVP, NIAID	L. L. Lubke	Biologist	LVP, NIAID	T. G. Schwan	Sr. Staff Fellow	LVP, NIAID
PI:	Claude F. Garon	Chief	LVP, NIAID																						
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	L. L. Lubke	Biologist	LVP, NIAID																						
	T. G. Schwan	Sr. Staff Fellow	LVP, NIAID																						
COOPERATING UNITS (If any) LPVD, RML, NIAID; LMSF, RML, NIAID; LICP, RML, NIAID Department of Medical Microbiology - Stanford University School of Medicine																									
LAB/BRANCH Laboratory of Vectors and Pathogens																									
SECTION Structural Pathobiology																									
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD																									
TOTAL MAN-YEARS: 3.6	PROFESSIONAL: 1.2	OTHER: 2.4																							
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																									
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <p> This project serves to consolidate and to expand our efforts at identifying, localizing, and characterizing genes and gene products which are important in host pathogen relationships. Current biological and serological techniques for demonstrating infections by <i>Borrelia burgdorferi</i> can be inconclusive. In order to monitor Lyme borreliosis, we developed a rapid and sensitive assay for <i>B. burgdorferi</i> antigens in infected hosts. Polyclonal rabbit antisera were raised against membrane vesicles and an 83 kDa vesicle-associated protein band that was purified from <i>in vitro</i> <i>B. burgdorferi</i> cultures. Immunoglobulin G (IgG) antibodies were recovered from these sera and tested for a species-specific reaction with several geographically diverse <i>Borrelia</i> isolates by immunoblot analysis. Parlodion-coated electron microscope grids were activated with anti-vesicle F (ab')₂ fragments and then incubated with confirmed or experimental sources of spirochetal antigens. Such sources included cultured spirochetes; spirochete culture supernatants; samples of urine, blood, or serum from mice, dogs, and humans; triturates of <i>Ixodes</i> ticks; and bladder, spleen, liver, kidney, heart, or brain tissues from infected or control mice. Captured antigens were assayed by immune electron microscopy by using anti-83 kDa IgG antibodies and protein A colloidal gold conjugates. The results indicated that <i>B. burgdorferi</i> appears to shed surface antigens which are readily detectable in urine, blood, and several organs from infected hosts. Such antigens were detectable in mouse urine at dilutions exceeding 10⁻⁶. Intact spirochetes were frequently observed on grids incubated with blood, spleen, or bladder preparations, and <i>B. burgdorferi</i> was reisolated from the urinary bladders of all experimentally infected mice. These results indicated that <i>B. burgdorferi</i> antigens arise in a variety of host materials. Such antigens can be captured and identified with specific polyclonal antibodies, providing a sensitive assay for monitoring and studying Lyme borreliosis. </p>																									

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00492-05 LVP

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Basis for Infection by *Borrelia burgdorferi*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Tom G. Schwan	Sr. Staff Fellow	LVP, NIAID
OTHERS:	W. J. Simpson	Visiting Associate	LVP, NIAID
	K. L. Gage	IRTA Fellow	LVP, NIAID
	M. E. Schrumph	Bio. Lab. Tech. (Micro)	LVP, NIAID
	R. H. Karstens	Bio. Lab. Tech. (Micro)	LVP, NIAID
	W. Burgdorfer	Scientist Emeritus	LVP, NIAID

COOPERATING UNITS (If any)

Microbial Diseases Laboratory, California Department of Health, Berkeley (Jane Wong)

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Arthropod-borne Diseases

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

1.7

OTHER:

1.7

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..)

The objectives of this project are to 1) use recombinant DNA techniques to express *Borrelia burgdorferi* specific antigens to improve the serodiagnosis of Lyme disease, 2) determine immunogenic properties of spirochetal components responsible for infection, and 3) determine the role of antigenic variation as an underlying mechanism for persistent spirochetal infection in hosts in spite of significant antibody response to infection.

Efforts to improve the serodiagnosis of Lyme disease have been successful through the cloning and expression of a 39 kDa of *B. burgdorferi* that is specific and highly immunogenic in both experimentally infected animals and humans naturally exposed to the Lyme spirochete. Kits containing this antigen are under current review by the FDA and should be available within the next year.

Preliminary studies exploring *in vivo* antigenic variation in mice have been completed. Adult laboratory mice, *Mus musculus*, were shown to be suitable experimental animals for this work. Western blot analysis of immune serum from each of 16 persistently infected mice demonstrated that spirochetes used to infect the mice reacted differently when compared with the spirochetes subsequently reisolated from the mice, demonstrating for the first time that changes in antigenic reactivity had occurred in the spirochete populations during persistent infection.

We have now infected *Ixodes* ticks with cloned populations of *B. burgdorferi* and these ticks will be used to infect subhuman primates to explore these animals as a model for Lyme disease and *in vivo* antigenic variation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00552-03 LVP

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathobiology of Bacterial Toxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	Witold Cieplak, Jr.	Sr. Staff Fellow	LVP, NIAID
OTHERS:	C. Cluff	IRTA Fellow	LVP, NIAID
	R. Messer	Microbiologist	LVP, NIAID

COOPERATING UNITS (If any)

Smith Kline Beecham (Lobet); Praxis Biologics (Cowell)

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Molecular Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.8

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to investigate the structure-function relationships and genetic regulation of bacterial ADP-ribosylating toxins using site-directed mutagenesis and recombinant expression systems. Studies of the amino acids essential to the enzymatic/toxic activity of the diarrheagenic toxin from enterotoxigenic *Escherichia coli* (heat-labile enterotoxin) have identified several residues that are likely to be involved in the active site of this toxin. These residues appear to be suitable sites for efforts directed at creating inactive holotoxoid forms of the heat-labile enterotoxin and perhaps of the homologous toxin from *Vibrio cholerae* (cholera toxin) for vaccine use.

Additional studies have allowed the detection of an intrinsic regulatory mechanism associated with the expression of the pertussis toxin gene. This mechanism, which is continuing to be defined, imparts negative (or down) regulation of the first gene in the operon. Precise definition of the mechanism may be of some importance in the construction of *Bordetella pertussis* strains which are pertussis toxin hyper-producers for vaccine purposes. Other collaborative studies have characterized the biologic and enzymatic properties of mutant forms of pertussis toxin and have permitted the precise evaluation of their suitability for vaccine use.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00553-03 LVP
PERIOD COVERED October 1, 1990, to September 30, 1991		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biology and Immunology of Pathogenic <i>Campylobacter</i> spp.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Witold Cieplak, Jr.	Sr. Staff Fellow LVP, NIAID
OTHERS:	Michael E. Konkel	IRTA Fellow LVP, NIAID
	Susan G. Smith	Microbiologist LVP, NIAID
	David Meade	Microbiologist LVP, NIAID
COOPERATING UNITS (if any) Stanford University Medical School (Tompkins); University of Arizona College of Veterinary Sciences (Joens)		
LAB/BRANCH Laboratory of Vectors and Pathogens		
SECTION Molecular Pathogenesis Unit		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided..) <p> The primary focus of this work involves elucidation of the pathogenic mechanisms associated with enteritis produced by <i>Campylobacter jejuni</i>. To date, very little is known concerning the virulence factors produced by this organism. Various pathogenic properties including, invasiveness and toxin production have been attributed to <i>C. jejuni</i>. However, the evidence for existence of such attributes is highly tentative. We have investigated both of these potential virulence factors using a variety of techniques. We believe we have established firmly, and for the first time, that <i>C. jejuni</i> is capable of entering and proliferating within cultured epithelial cells of intestinal origin. Further, <i>C. jejuni</i> can penetrate polarized epithelial cells in culture with kinetics that approach that of invasive strains of <i>Salmonella</i> and <i>Yersinia</i>. These studies suggest that adherence and internalization as observed <i>in vitro</i> are relevant pathogenic mechanisms associated with <i>C. jejuni</i> infection. We have characterized in detail the factors that influence these interactions and have further identified a fibronectin-binding protein on the surface of <i>C. jejuni</i> which may mediate attachment to intestinal epithelial cells. The amino-terminal amino acid sequence of this protein has been determined and has been used to generate oligonucleotide probes which are in turn being used to identify cloned gene sequences in a <i>C. jejuni</i> genomic library. We have also identified several new proteins that are synthesized by <i>C. jejuni</i> during intracellular residence. These appear to include members of the family of stress-response proteins. Other studies have failed to reveal any evidence for the production of enterotoxin-like molecules by <i>C. jejuni</i> as judged by a variety of immunologic, enzymatic, or biologic assays. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00554-03 LVP

PERIOD COVERED

October 1, 1990, to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Characterization of Microbial Genes and Nucleic Acid Molecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	Claude F. Garon	Chief	LVP, NIAID
OTHERS:	R. T. Marconi	IRTA Fellow	LVP, NIAID
	D. W. Dorward	Staff Fellow	LVP, NIAID
	L. L. Lubke	Biologist	LVP, NIAID
	D. S. Samuels	IRTA Fellow	LVP, NIAID

COOPERATING UNITS (If any)

None

LAB/BRANCH

Laboratory of Vectors and Pathogens

SECTION

Structural Pathobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MAN-YEARS:

1.9

PROFESSIONAL:

1.3

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lyme borreliosis is now the most common arthropod-borne disease in the United States. *Borrelia burgdorferi*, the causative agent, has been isolated from humans, mammals, birds and arthropods and is cultivable in the laboratory. Careful structural analysis of the DNA content of several early passage isolates has revealed, in addition to a 1,000 kb linear genome, a unique mixture of terminally cross-linked linear and covalently-closed, circular DNA molecules ranging in size from 2 to 50 kilobases in length. Often DNA patterns appear to differ among isolates and to vary during laboratory passage.

While all of the linear species were shown to rapidly reanneal to linear duplexes after alkaline denaturation, single-stranded circular molecules measuring twice the length of the linears could be produced by treatment with methyl mercury, glyoxal and urea prior to mounting for electron microscopy. This rapid reannealing mediated by the terminal sequences of the molecule was used in a unique ion exchange column chromatography system to purify terminal, restriction fragments from any which originate internally. Purified terminal fragments could then be compared and characterized further by conventional methods. The objective of this project, therefore, is to define the genetic capacity of *B. burgdorferi* and other vector-borne pathogens in sufficient detail to begin the process of mapping those genes and gene products which may be important in pathogenesis.

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1991 Annual Report
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1990 to September 30, 1991

Introduction

The branch provides all services necessary to the professional staff in the pursuit of their investigations. Fiscal support includes budget management, procurement, and initiating payments and follow-up on financial obligations related to purchases, contracts, staff and official guest travel, and expenses for conferences held at RML. Other support covers the following areas: personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of the power plant, and full maintenance and minor laboratory renovations in every area except electronics.

We have continued to upgrade the facilities in the Animal Care Section. Five animal procedure rooms have been established and equipped with appropriate furnishings and scavenging systems for volatile anesthetics. Ten additional procedure rooms are planned. We have replaced much of the old galvanized steel equipment and are replacing old worn out caging, and increasing the use of microisolator caging and clean air work stations. New group caging has been installed for the nonhuman primates. This allows for two-thirds of the primate colony to be housed in large groups. New chemical resistant flooring has been put down in three washrooms and the big walk-in autoclave has been converted from a gravity type to a vacuum type operation.

The Maintenance Section renovated two old rooms in building 3 to provide additional space for scrapie research; as well as several other minor projects for the Operations Branch, LMSF, LPVD and LICP.

Major changes were made in the mechanisms for processing procurement actions. The Institute's computerized Acquisition Management and Budget Information System (AMBIS) was installed at RML and a purchasing agent was hired to be the ordering official for procurement actions. Requisition worksheets are now submitted electronically by requestors and the information is then transferred electronically by the Purchasing Agent to the purchase orders.

Personnel in the Operations Branch have been involved in varying degrees to host three different meetings. On July 12 - 15 the NIAID Task Force on Microbiology and Infectious Diseases met in Hamilton. A meeting entitled "The Molecular Immunology of STD's", sponsored in part by LMSF and LICP was held at the RML on July 30 - August 3. The Second Annual Meeting of Grantees Studying the Pathogenesis of Lyme Boreliosis was held at RML on September 12 - 14.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of over 3.5 million. Payroll is not included in this figure. It covers only the purchase of services, supplies, and equipment used in the operation of the laboratories. Timekeeping and submission of the payroll are also handled in this unit.

Personnel initiates civil service and commissioned officer personnel actions, and advises on personnel matters. This department is also charged with operation of the Job Training Partnership Act in association with the local Montana State Employment Office when the program is active. Also handled by Personnel are persons under the following programs: Stay-in School, Summer Aides, Student Volunteers, Special Volunteers, Visiting Program, and students studying for advanced degrees.

Most of the biological media used in research laboratories is prepared in a media kitchen by a technician.

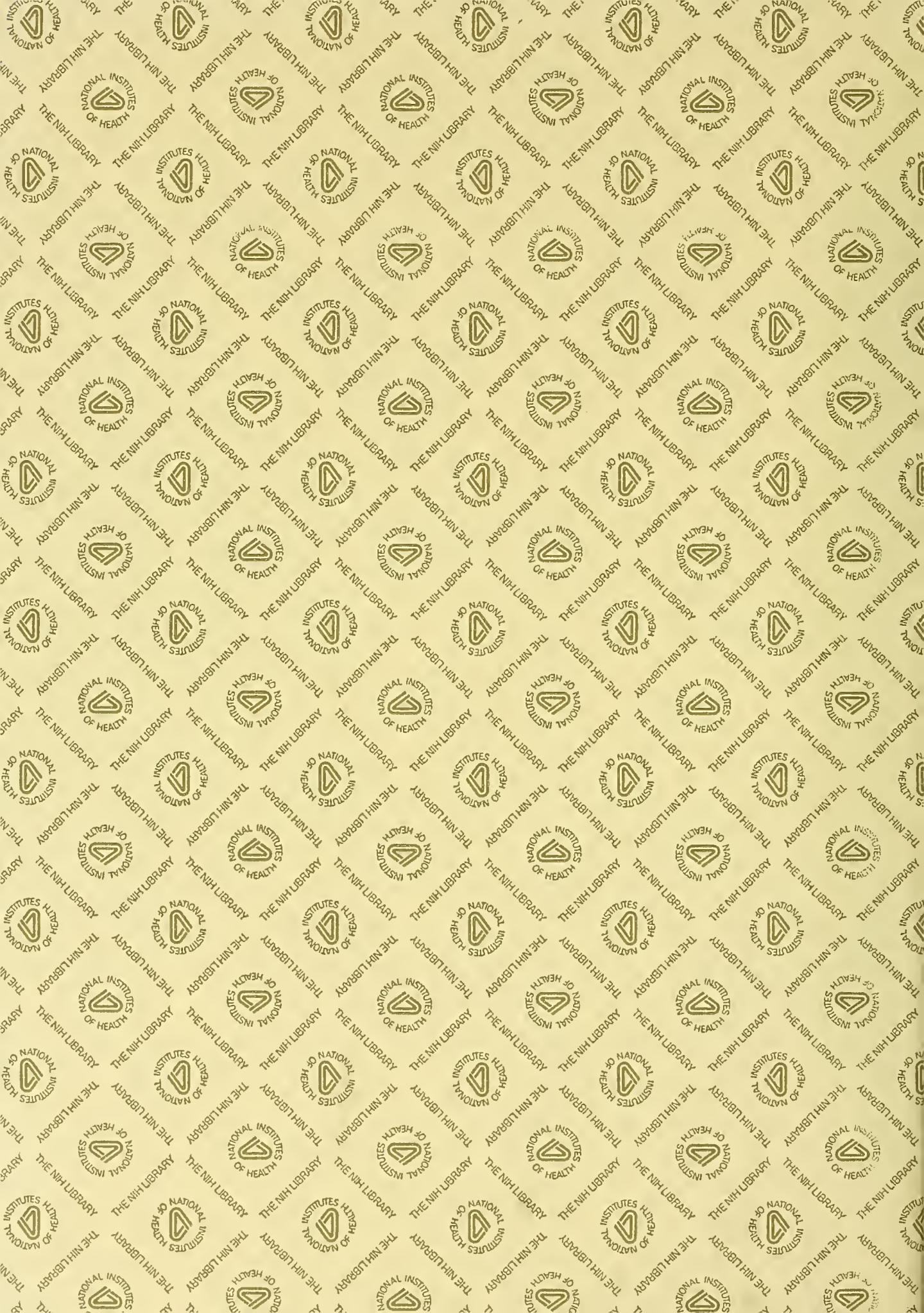
The Graphic Arts Department provides full professional services to the laboratory staffs with the exception of medical artistry.

The library provides a full range of services for the RML staff, i.e., selections and acquisitions, cataloging, circulation, inter-library loans, reference and bibliographic services, computerized data base searches, and preparing periodicals for binding.

The Animal Care Section raises 2 strains of guinea pigs, 21 strains of mice, 7 strains of hamsters, a colony of grasshopper mice, a colony of peromyscus and a colony of microtus. They are raising 84,800 animals annually for research from an average pool of 3,000 breeding stock (approximate figures). An additional 4000 to 5000 animals are purchased annually from outside sources, including mink, rabbits, rats, mice, hamsters and guinea pigs. After rearing, care is provided for these animals while they are under experiment. The most recent survey indicates an average daily holding of 16,000 small animals. An experimental colony of 39 cynomolgus macaques, as well as 2 - 3 horses and 2 sheep are also maintained in the animal facilities.

The Chief of the Branch is responsible for labor management work and administering the technical aspects of the contracts for Security, Custodial, operation of the Boiler Room and the Glassware Unit with the respective private contractors. Security is provided by a guard on duty every night and all day on weekends and holidays. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Boiler Room operation provides heat, steam, compressed air, vacuum, and emergency power to the entire laboratory complex. Glassware is cleaned and sterilized in the Glassware Unit for reuse in the laboratories.

The Maintenance Department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning, and refrigeration, including ultra-low temperature boxes. With exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.

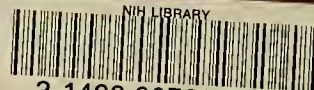




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